LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING

STATE INSTITUTE OF HEALTH AND FAMILY WELFARE
UTTAR PRADESH

ACKNOWLEDGEMENT

GUIDANCE

Shri. Partha Sarthi Sen Sharma, I.A.S
Principal Secretary,
Department of Medical Health and Family Welfare,
Government of Uttar Pradesh

DIRECTION AND LEADERSHIP

Dr. Rajaganapathy R., IAS,
Director, SIHFW, Uttar Pradesh & Director (Administration)
Medical and Health Services, Uttar Pradesh

AUTHOR / CONTRIBUTOR

Dr Suresh Babu MD
Professor, Department of Pathology
King George Medical University
Lucknow, UP, India.

Co- Contributors:

Dr Mala Sagar MD
Professor
Department of Pathology
KGMU Lucknow, UP, India.

Dr Madhu Kumar MD
Professor
Department of Pathology
KGMU Lucknow, UP, India.

Dr Rashmi Kushwaha MD
Professor
Department of Pathology
KGMU Lucknow, UP, India.

Dr Archana Verma
GM – Training,
National Health Mission,
Uttar Pradesh Unit, Lucknow

Dr Vikasendu Agarwal
Joint Director,
Incharge- Communicable Diseases,
Directorate of Medical & Health Services,
UP, Lucknow

Dr Sheetal Verma MD
Add. Professor
Department of Microbiology
KGMU Lucknow, UP, India.

Dr Chitra Suresh,
Joint Director,
UP State Aids Control Society
(UPSACS) , Lucknow

Mr. Suneel K Yadav
Chief Pharmacist,
Dr. S P M Hospital,
Lucknow

Mahesh Prasad
Senior Lab Technicians
Urban Community Health Center
Indira Nagar, Lucknow

Santosh Kumar Jauhari
Lab Technicians
Epidemic Branch, Swasthya Bhawan,
Lucknow

SIHFW Editorial Board :

Dr. Mahesh Nath Singh
Assistant Professor

Mudasser Ahmed
Assistant Professor

Dr. Diwakar Yadav
Assistant Professor


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

FOREWARD

Shri Partha Sarthi Sen Sharma
I.A.S
Principal Secretary
Department of
Medical Health and Family Welfare
Government of Uttar Pradesh

Medical lab technicians are essential in the healthcare field. They work behind the scenes, performing different laboratory tests and analyses that assist in the diagnosis, monitoring, and treatment of diseases. Role of Laboratory Technician is extremely crucial towards improving public health and disease prevention.

Medical Laboratory Technician's job responsibilities revolve around ensuring the accuracy and precision of results. They are responsible for sample collection and processing, which involves following strict protocols to prevent contamination and ensuring that the sample represents the patient's condition. Once the samples are collected, they perform tests according to established procedures.

It is essential that they are proficient in using lab equipment and are familiar with various testing methodologies. It is crucial to record and analyze data accurately to detect anomalies and report them to the appropriate authorities. Maintaining lab equipment is also one of their duties, so they need to have technical skills to troubleshoot and repair equipment when necessary.

Additionally, they are in charge of quality assurance in lab procedures, which involves skill up gradation, identifying areas for improvement, and implementing changes to ensure reliable and precise results. As a medical lab technician, attention to detail is critical. Minor errors can have adverse effects on patient care and public health.

Considering the important and complex role of Medical Laboratory Technician, this module on LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING becomes exceedingly important from medical intervention perspective in the state.

I congratulate the faculties of State Institute of Health & Family Welfare, Uttar Pradesh and subject matter experts for developing an impactful module that will enhance the quality of health care and improve OPDs footfall at PHCs/CHCs.

(Partha Sarthi Sen Sharma)


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

MESSAGE

Dr. Brijesh Rathor

Director General
Medical and Health Services
Uttar Pradesh

Inaccurate results in medical laboratory science can have grave consequences for patient care and public health. Medical lab technicians play a vital role in ensuring accurate and precise results by following stringent procedures and protocols. Inaccurate results can lead to incorrect diagnosis, delayed treatment, and even patient harm. Moreover, false negative results can contribute to disease outbreaks and epidemics. This highlights the criticality of accuracy and precision in medical lab science.

Medical lab technicians use various measures to ensure accurate and precise results. They follow established procedures, standards, and guidelines, regularly calibrate and maintain lab equipment, and conduct quality control measures to detect and prevent errors. They also employ critical thinking and analytical skills to interpret and analyze data.

In summary, medical lab technicians play a crucial role in public health and disease prevention. They ensure accurate and precise results, which are essential for effective patient care and population health monitoring.

Considering the above stated facts, this module on LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING, is an excellent document developed by faculties at State Institute of Health & Family Welfare, Uttar Pradesh with the help of subject matter experts and this module will ensure that Medical lab technicians are given latest skills and knowledge to help them in performing their duties to the optimum level.

I congratulate the team of State Institute of Health & Family Welfare, Uttar Pradesh and subject matter experts for such a commendable job.

(Dr. Brijesh Rathor)


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

MESSAGE

Dr. Shailesh Kumar Srivastav

Director General Family Welfare
Directorate of Family Welfare
Uttar Pradesh

Primary Health Centers (PHCs) enable cost-effective, accessible and universal coverage of health to the individual and community. These PHCs are responsible to provide both preventive as well as basic curative services in poor rural areas.

Good functioning of PHC plays an important role in utilization of its services by the masses. Laboratory service is recommended as an important component for good functioning of PHC. It is an important issue because PHC laboratory is the only diagnostic facility for people living in rural areas of developing countries like India.

Laboratory services could play an important role in maximizing the PHC performance. Higher level of laboratory services in PHC could help in getting more visits in the OPD. The training of existing LTs could be a cost-effective approach in resource-constrained settings to maximize the returns from the existing medical workforce in PHCs.

Considering the above stated facts, and the importance of Laboratory Services in the public health system, this module on LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING, developed by the faculties of State Institute of Health & Family Welfare, Uttar Pradesh with the help of Subject Matter Experts, has provided a comprehensive, coherent and insightful practice based knowledge for Medical Laboratory Technicians.

I congratulate the best to the faculties of State Institute of Health & Family Welfare, Uttar Pradesh and subject matter experts for such a commendable job.

(Dr. Shailesh Kumar Srivastava)


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

MESSAGE

Dr. Narendra Agarwal

Director General-Training
Medical and Health Services
Uttar Pradesh

Improving the efficacy of in-service trainings of Medical laboratory professionals in the state is possible even if challenged by many factors. Some of those are specific to the training approach adopted, while others are related to human resources management policies.

In order to have a robust public health system knowledgeable, skilled and motivated Medical Lab Technicians are exceedingly important. The transfer of theoretical knowledge to practical skills at laboratory level is extremely necessary. For long-term retention of knowledge and for laboratory staff motivation, it is important that regular and periodic training are carried out to enhance and upgrade medical laboratory technician skills.

The numerous efforts in strengthening the laboratory systems in the state can be successful with an effective training approach that is adopted and ingrained into a broader strategy for human resource management at the facility level.

Keeping the above facts in mind, State Institute of Health & Family Welfare, Uttar Pradesh with the help of Subject Matter Experts has developed an extensive and up to date module on LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING addressing all the underlying nuances thus providing a comprehensive, coherent and insightful module for medical laboratory technician.

I applaud the faculties of State Institute of Health & Family Welfare, Uttar Pradesh and subject matter experts for such a commendable job.

(Dr. Narendra Agarwal)


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

ACKNOWLEDGEMENT

Dr. Rajaganapathy R.
I.A.S
Director
State institute of
Health & Family Welfare
Government of Uttar Pradesh

Medical Laboratory Technician are unsung heroes in the field of healthcare, working with great dedication to ensure precise diagnosis, keeping a close watch on the outbreak of diseases, and contributing significantly towards health and disease prevention is the cornerstone of public health work. Their competence, meticulousness, and devotion to quality control play a critical role in maintaining the efficacy of the healthcare systems.

Medical Laboratory Technician are essential in the healthcare field. They work behind the scenes, performing different laboratory tests and analyses that assist in the diagnosis, monitoring, and treatment of diseases. Role of Laboratory Technician is extremely crucial towards improving public health and disease prevention.

In the light of these above stated facts, it is imperative that the skills and knowledge of lab technicians in Provincial Health & Medical Services in Uttar Pradesh is up to par to tackle the public heath challenges in the state. This module on LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING, defines and facilitates the laboratory protocols alongside enhancing capacities of Laboratory Technicians.

I acknowledge the sincere efforts made by the faculties of State Institute of Health & Family Welfare, Uttar Pradesh and by Dr. Suresh Babu-MD, Professor-Department of Pathology, King George Medical University, Lucknow, UP, India and his team in developing such a comprehensive, coherent and insightful module for Laboratory Technicians.

(Dr. Rajaganapathy. R)


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

PREFACE

This laboratory manual is a selected review of the main laboratory techniques can be used for the training of laboratory technicians working in the Health department, medical education department as well as antpathological laboratory in Government of Uttar Pradesh and any other medical institutes. All the tests presented in the manual correspond to the disease most found in our state of Uttar Pradesh. The techniques presented in the manual are those most appropriate to use in the camp laboratories and are feasible to standardize in all over the state Labs. Most of all equipment and supplies can be purchased and maintained in State Institute of Health and Family welfare, Uttar Pradesh. This manual is intended to prepare displaced persons who are the expert as well as special invitee Dr Suresh Babu Professor in Pathology King George Medical University Lucknow, Uttar Pradesh and maintain laboratories as well as train additional laboratory technicians on their own and consider from different references.


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

TABLE OF CONTENTS


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादृते न मुक्तिः

CHAPTER - 1

HEALTH AND DISEASE

WHAT IS THE HEALTH ?

Physical, mental, social and spiritual well-being of individual as well as community is the called health .

WHAT IS A HEALTHY PERSON?

Somebody, who feels well has no pain, eats well and sleeps well. A healthy child grows well. A healthy adult has normal activity.

WHAT IS A DISEASE?

Anything that disturbs the normal function of the body., Or any discomfort of body is the disease. A person who has a disease will feel various symptoms; for example: headache, abdominal pain, chills. These symptoms are only caused by the disease. They are not the diseases.

CAUSE OF DISEASE:

1) A Disease can be caused by a lack or an excess of something, for example of food.
   - ► Lack of food for child : no growth, edema, weakness…
   - ► Lack of essential vitamins : eye problems (vitamin A), skin disease (vitamin B)….
   - ► Drinking too much alcohol: liver problems
2) A disease can also be caused by infectious agents. Infectious agents are parasites, bacteria, viruses or other small living things that you can not see with your eyes that enter our body from the outside and cause diseases.
   - ► Some examples of parasites are worms and the parasite that causes malaria
   - ► Some examples of bacteria that causes diseases are the bacteria that cause tuberculosis and the bacteria that cause wounds to get infected (cause pus in wound).
   - ► Some viruses cause diseases like the common cold and measles.
3) A disease can also be caused by abnormality inside the body such as diabetes, cancer…

WHAT IS THE ROLE OF A DOCTOR OR A MEDIC?

The doctor or the medic will examine the patient to find clinical signs of the disease. For example; big liver, hard belly, noise in the lungs, fast pulse….

For some diseases, examining only the clinical signs and symptoms is enough for a medic to treat the patient. Sometimes it is not enough, so they need the help of a laboratory for other examinations before giving treatment.

WHAT IS THE ROLE OF THE LABORATORY?

The laboratory will find the infectious agents that cannot be seen only by eyes, because they are very small in size. To find them, the laboratory uses a microscope.

The laboratories can also check for some bodily substances, especially in blood and urine. For example: some kind of sugar in the blood or protein in urine.

While doctors and medics examine the whole body, laboratory technicians examine only samples from the body such as blood, urine, sputum, and stool. These samples are also called specimens.

PATIENT	DOCTOR	LABORATORY
Symptom ® # *Example:(fever)*	***Clinical Signs*** ® *(Big liver)* #	***Infectious Agents*** *(Malaria)* ß#
Healthy	¬ # **Treatment**	¬ # **Result**

CHAPTER - 2

JOB DESCRIPTION

A. LABORATORY TECHNICIANS

1.	Collect the specimen:	-	Sputum/Blood/Urine/Body fluid/Others
2.	Prepare the specimen:	-	Thin and thick blood smears
			Dry and wet sputum smear
3.	Make the staining:	-	As per need of test
4.	Examine for :	-	Malaria parasites
		-	TB Bacteria
		-	Lung parasite (Paragonimus)
		-	Microfilaria parasite
		-	Hemoglobin or hematocrit measurement
		-	Blood grouping
		-	HIV
		-	Hepatitis B

5. Record each specimen with the result in the register book.
6. Return the result to the medic or nurse. The laboratory technician is responsible for the result that he/she gives. In emergency cases, he/she must give the result directly to the medic as quickly as possible.
7. Keep the laboratory clean:

   Clean the room: Tables, chairs, shelves, floor, and boxes

   Clean the laboratory equipment

   Clean the old slides

   Properly dispose of all waste
8. Maintain the microscope properly; dry the desiccant (silica gel) for reusing.
9. Regularly check the stock of reagents, equipment, and items and fill out the inventory (stock) and the order form that you will give to the in-charge.
10. Do the statistics at the end of the month and fill in the laboratory statistic form.
11. Collect the slides for quality control every month and send to the lab supervisor.

QUALITIES OF THE LAB TECHNICIANS

Conscientious, Honest, Neat, and Reliable

Lab technicians should be aware that the patient's life depends on the results he/she gives.

B. LABORATORY SUPERVISORS

The supervisors should be able to:

1. Perform all the laboratory activities described in the laboratory worker's job description.
2. Communicate:
   - 2.1 Between the laboratory and the other departments (OPD, IPD...):
     - Discuss the result of all IPD patients with the medics.
     - With the medic, organize a meeting if there is a problem to solve.
     - Teach the nurses and medics for the proper collection of specimens, especially thin and thick blood smear, in case the lab technicians cannot do it on their own. Bad smears can lead to wrong results, so the lab technician supervisor should show the medics and nurses how to make a good smear and explain that the quality of the result depends on it. However, blood collection should be done by the lab technician as much as possible.
   - 2.2 Among the laboratory staff:
     - Organize a meeting with the staff if there is a problem.
     - Organize the staff duties.
   - 2.3 With the NGO laboratory technician in-charge:
     - Discuss the problems met during the month.
     - Give new ideas to improve the work.
     - Complete and send the statistic forms, quality control form, quality control slides, and laboratory order form on time.
     - Discuss about the monitoring record.
3. Monitor the laboratory activities:
   - 3.1 Check the collection of the specimens:
     - Blood
     - Sputum
   - 3.2 Evaluate all the techniques done in the laboratory:
     - Thin and thick smears
     - Giemsa staining
     - TB smears
     - TB staining
     - Paragonimus smears
     - Hemoglobin or hematocrit measurement
     - Blood grouping
     - HIV tests
     - Hepatitis B tests
   - 3.3 Control the registration of the results in each book and on forms:
     - Malaria book
     - TB book
     - Hemoglobin or hematocrit book
     - Blood transfusion book
     - Lab request forms
     - Statistics forms
     - Quality control forms
   - 3.4 Confirm the results from the lab technicians if they are not sure.
   - 3.5 Control the laboratory inventory and ordering system:
     - Recheck the inventory form done by the lab technician
     - Realization of the laboratory order
   - 3.6 Do quality control of malaria and TB slides.
   - 3.7 Fill out general monitoring records.
   - 3.8 Supervise the cleaning and maintenance of the lab items and the laboratory room

CHAPTER - 3

SAFETY IN THE LABORATORY

The main accidents associated with medical laboratory work are:

- **INFECTIONS**
- **BURNS**
- **WASTES**
- **CUTS**
- **HARMFUL EFFECTS OF TOXIC CHEMICALS**

INFECTIONS

Laboratory specimens are often infectious:

- ► Sputum of TB patients contains bacteria that may infect the lab technicians.
- ► Urine of patients with urinary tract infection (UTI) contains bacteria. If the lab technician touches this urine, then touches his/her mouth or his/her eyes, he/she may get an infection.
- ► Stool may contain parasites such as roundworm eggs. If swallowed, they will give roundworm infection.

To avoid this, you must:

- Put a mask on before smearing a sputum specimen.
- Put gloves on before you work with any specimens or when cleaning equipment.
- Always wash your hands after handling specimens.
- Be very careful with needles, blood lancets, and dispose of them properly.

Using these safety methods is called **UNIVERSAL PRECAUTIONS**. *If you follow this, you will protect yourself from the diseases that can infect and kill you. Anytime that you handle any specimens for laboratory tests, you must treat them as if they were infectious. Do not treat only the specimens that you think are infectious. You must assume that every patient has an infection. This way, you will protect yourself from the possibility of getting an infection.*

CUTS

- Lab work requires some glassware: slides, beakers, cylinders, pipettes, bottles.
- You must be careful with cracked glassware, especially when you wash slides.

BURNS

- Different kinds of alcohol are used in the laboratory. All of them are flammable.
- Acid alcohol, used in TB tests, contains acid that causes burns on skin and clothes.

HARMFUL EFFECTS OF TOXIC CHEMICALS

- All the chemicals used in the laboratory are toxic if swallowed.
- Mouth pipetting should be avoided. Never mouth pipette for acid.
- Avoid skin contact with xylene, and do not breathe it.

WASTES

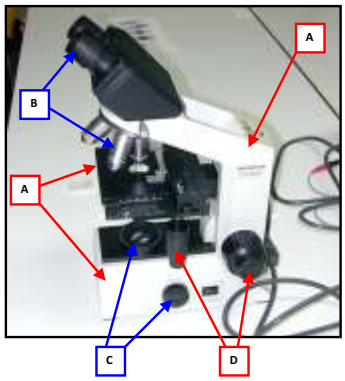
- Before washing used slides (especially unstained slides) or specimen containers, throw the wasted specimen into the latrine. Then soak these slides and containers with disinfectant (solution used for destroying the infectious agents), such as chlorine and hypochlorite.
- Old slides and used blood lancets must be collected in closed bottles or boxes (or old infusion bottles) and thrown into the latrines or wasted wells.

NEVER LEAVE USED SLIDES OR BLOOD LANCETS LYING ON THE FLOOR.

CHAPTER - 4

THE MICROSCOPE

Microscope is used to see something so small that you cannot see it with your eyes. A microscope can magnify the object up to 1000 times its real size.



The parts of the microscope can be classified into 4 systems:

A) THE SUPPORT SYSTEM

B) THE MAGNIFICATION SYSTEM

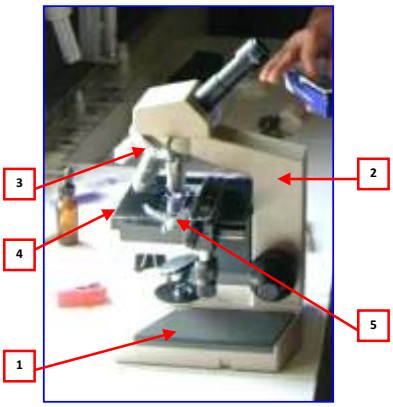
C) THE ILLUMINATION SYSTEM or THE LIGHT SYSTEM

D) THE ADJUSTMENT SYSTEM

A) THE SUPPORT SYSTEM

Consists of:

1. The foot
2. The body
3. The objective holder
4. The stage to place the slide or the object
5. The mechanical stage, which holds and controls the movement of the object slide



B) THE MAGNIFICATION SYSTEM

Consists of a system of lenses:

1. The eyepieces:

   They multiply the size of the object by 10.

2. The objectives:

   X10 objective multiplies the size of the object 10 times.

   X40 objective multiplies the size of the object 40 times.

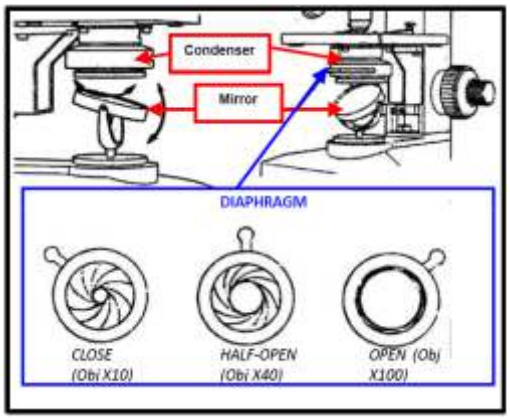
   X100 objective multiplies the size of the object 100 times.

   With the X100 objective, we use immersion oil on the slide.

Example: If the X40 objective and the X10 eyepiece are used, the total magnification will be 40 times (objective) multiplied by 10 times (eyepieces): 40 x 10 = 400. It means that the image will be magnified 400 times from the real eyesight image. Others are like that.

C) THE ILLUMINATION SYSTEM or THE LIGHT SYSTEM

Consists of:



1. The mirror that sends the light to the object.
2. The condenser that focuses the light.
3. The diaphragm, which is fixed within the condenser. It reduces or increases the light before passing through the condenser.

D) THE ADJUSTMENT SYSTEM

Consists of:

1. The coarse adjustment screw:

   This is the largest screw. It is used to adjust approximately.

2. The fine adjustment screw:

   This moves the objective more slowly. It is used to adjust perfectly.

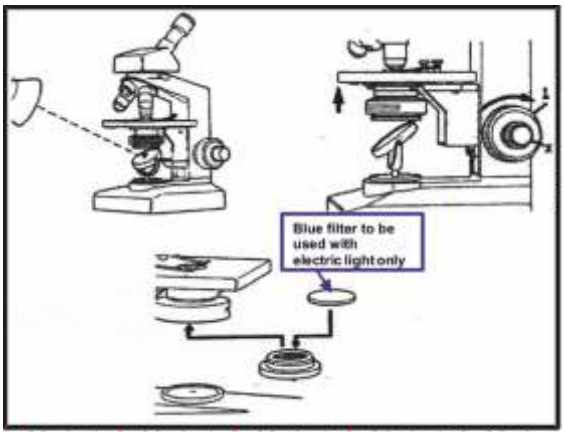
3. The condenser screw (some models do not have this):

   This is used to raise the condenser for greater light or to lower it to reduce the light:

   Using X10 objective: condenser down

   Using X40 objective: condenser halfway

   Using X100 objective: condenser up



4. The iris diaphragm lever:

   This is a small lever inside the condenser. It is moved to close or open the diaphragm to reduce or increase the intensity of light.

5. The screw of the mechanical stage:

   One screw moves the slide to the right or left.

   One screw moves the slide forward or backward.

ROUTINE MAINTENANCE OF THE MICROSCOPE

The microscope needs daily attention to keep it in good working order to ensure reliable laboratory results.

1. Remove the oil from the X100 objective with lens paper or soft tissue paper.
2. Clean the eyepieces with lens paper or soft tissue paper.
3. Clean the condenser, the mirror, and the support stage with a soft cloth.
4. Cover the microscope with a clean plastic or cloth cover in order to protect it from dust.
5. Put it in a sealed microscope box with an opened box of blue desiccant to protect from fungus developing on the microscope, especially on the lens surface.

Note: Desiccant must be renewed regularly by heating it. Its color will turn from pink to blue and be ready to be used again.

CHAPTER - 5

THE CELL

The cell is the basic unit of all living things. A cell is an important part of all animals or plants. It usually works together with other cells to form various organisms (it performs like a leaf, which is one component of an entire roof). Some organisms are formed of only one cell but others, like the human body or plants, have billions of cells.

Most of the organisms that cause diseases are one-cell living things, which are too small to be seen with the eye. Examples of these living things are *yeast, fungal ,Protozoal, Parasites, malaria and bacteria.*

Aside from these living things, there are free-living things, which are so small that we cannot see them even with an ordinary microscope. They are called Virus and they are not complete cells.

ONLY SOME OF EACH KIND OF THESE ORGANISMS CAN CAUSE DISEASE IN HUMANS

Although cells vary in shape, size, and function, they are similar in their main structure.

Commonly a cell consists of 2 major parts:

1. Nucleus
2. Cytoplasm

1. **NUCLEUS** is the inner part of the cell. Inside the nucleus, there is a very small material called Chromatin that acts like the brain of the cell.

2. **CYTOPLASM** is the fluid that surrounds the nucleus and is enclosed by a Cell membrane or/and Cell wall. Inside the cytoplasm, there are various components, which help the cell to perform its activities.

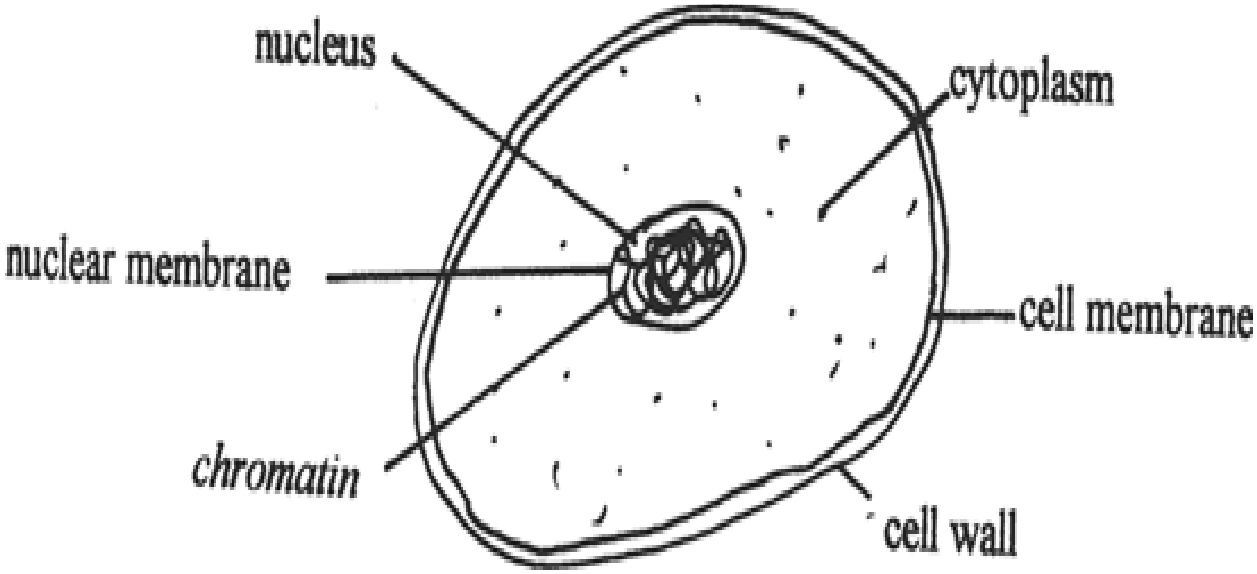


Fig 5. 1: Structure of a cell.

CHAPTER - 6

BLOOD CELL

Blood is a red liquid solution, which contains several different kinds of cells. These cells are:

1) Red Blood Cells

2) White Blood Cells

3) Platelets

1) THE RED BLOOD CELLS (RBC)

The RBC carries oxygen (good air) from lungs to all body tissue cells.

Size: 7 mm (micrometer or micron. 1000 mm = 1 mm)
It is seen about 7 mm by the x100 objective.

Shape: Round as a biconcave disc, occasionally slightly irregular.
Mature RBC is the only kind of cell in our body that has no nucleus.

Coloring by Giemsa stain : Periphery is pink-grey color
Center is pale pink-grey color or almost colorless

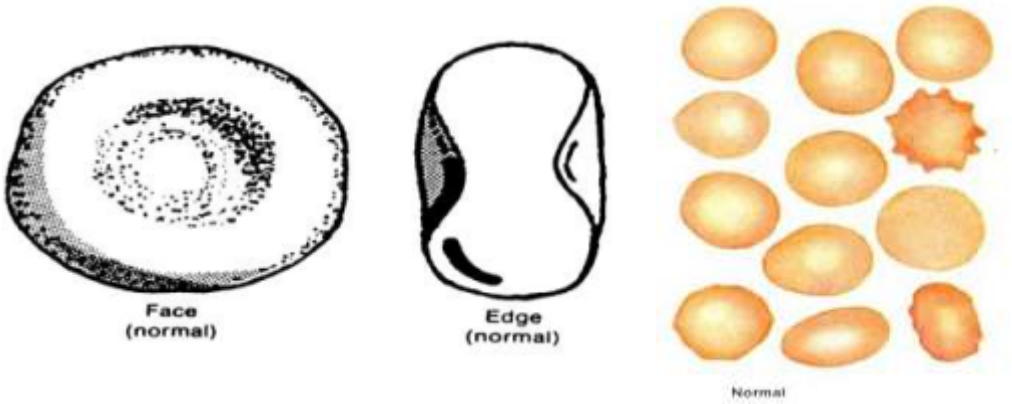


Fig 6. 1: Normal RBC as seen on a thin smear

2) THE WHITE BLOOD CELLS (WBC)

The WBC defends the body against infection. They act as the soldiers of our body. There are 5 main types of WBC, which differ in size, shape of the nucleus, color of cytoplasm and granules in the cytoplasm (granules look like spots).

We will study only 4 kinds of WBC:

2.1) Neutrophil

Size : 12 – 15 mm It is seen about 12 – 15 mm by x100 objective.

Shape : Round

Coloring by Giemsa stain:

Cytoplasm: Pinkish

Nucleus : Several (2 to 5) lobes, linked by strands of chromatin.
The chromatin forms a deep purple mass.

Granules: Mauve and very small, blue, numerous but separate.

For patients suffering from severe malaria the cytoplasm may contain brownish – black masses. This is malaria pigment (see figure 7. 20).

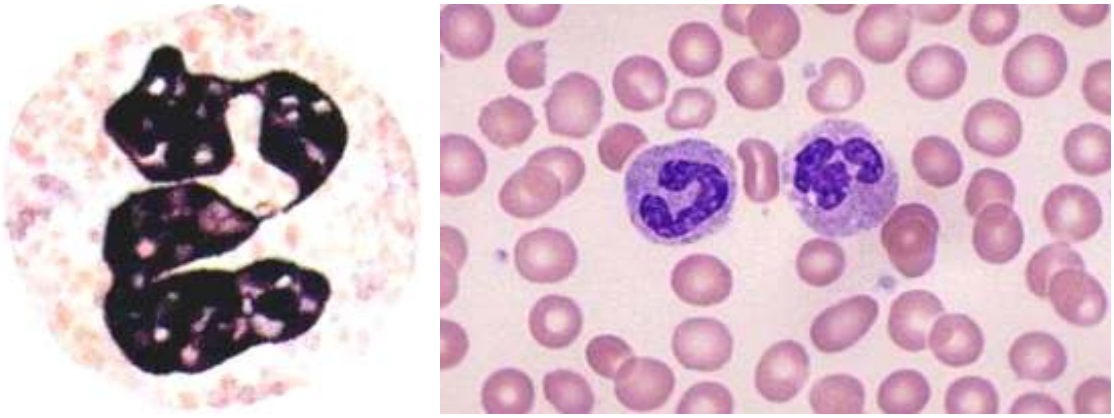


Fig 6. 2: Neutrophil

2.2) Eosinophil

Size: 12 – 15mm

Shape: Round

Coloring by Giemsa stain

Nucleus: Usually 2 lobes in a deep purple mass.

Granules: Large, round, orange, numerous and closely packed.
Sometimes the cell appears damaged, with scattered granules.

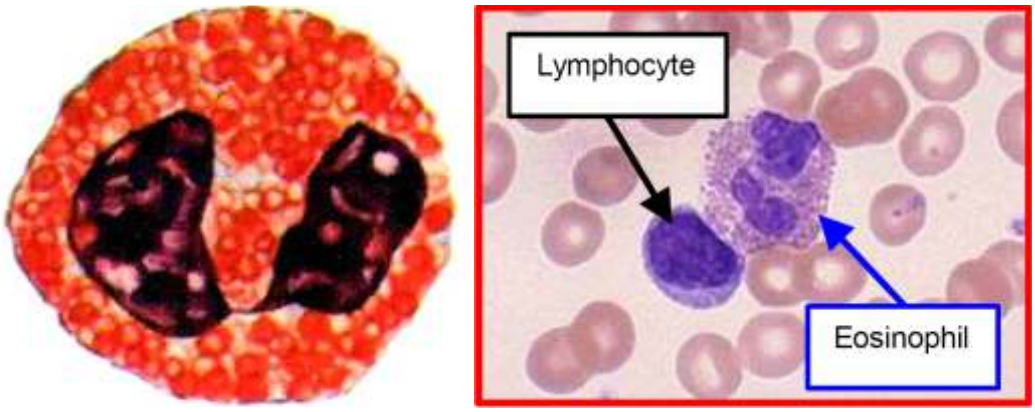


Fig 6. 3: Eosinophil

2.3) Lymphocyte

Size : 7 – 10 mm (They are smaller than the other kinds of WBCs)

Shape: Round

Coloring by Giemsa stain

Nucleus: One compact and large nucleus occupying most of the cell.
Chromatin is dark purple and dense.

Cytoplasm: Very little visible, pale blue without granules.

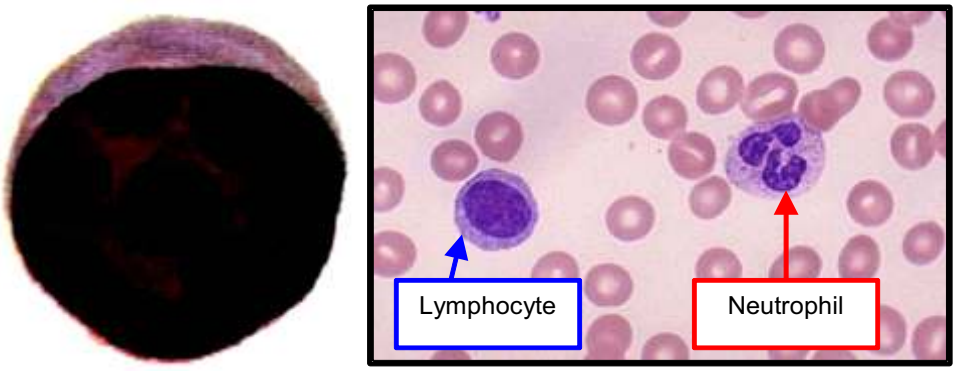


Fig 6. 4: Lymphocyte

2.4) Monocyte

Size: 15 – 25 mm (They are the largest kinds of WBCs)

Shape: Irregular

Coloring by Giemsa stain

Nucleus: Variable, often kidney – shaped.
Chromatin arranged in strands, pale mauve.

Granules: fine, dust – like, blue.

Cytoplasm: blue – gray, sometimes vacuoles are present inside.

For patients suffering from severe malaria the cytoplasm may contain brownish – black masses. This is malaria pigment.

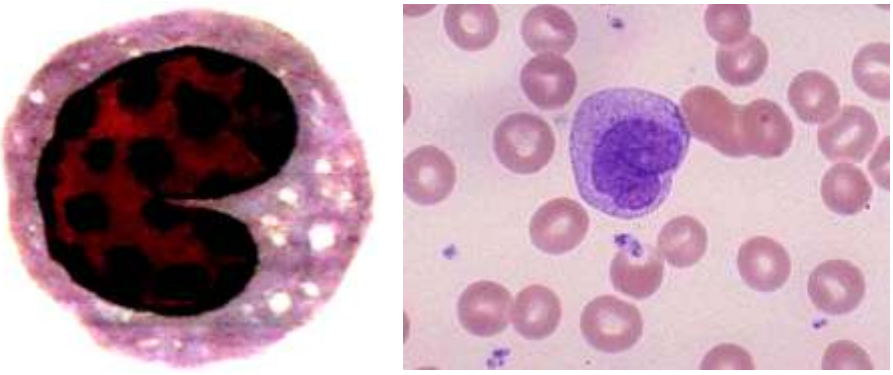


Fig 6. 5: Monocyte

3) THE PLATELETS (Plt.)

They are fragments of cells, which have an important function in the clotting of blood. Without platelets, bleeding from any scratches or cut wounds will not be stopped.

Size: 1 – 4 mm (It is seen about 1 – 4 mm by the x100)

Shape: various shapes (triangular, star – shape, oval, etc…)

Coloring by Giemsa stain: pink

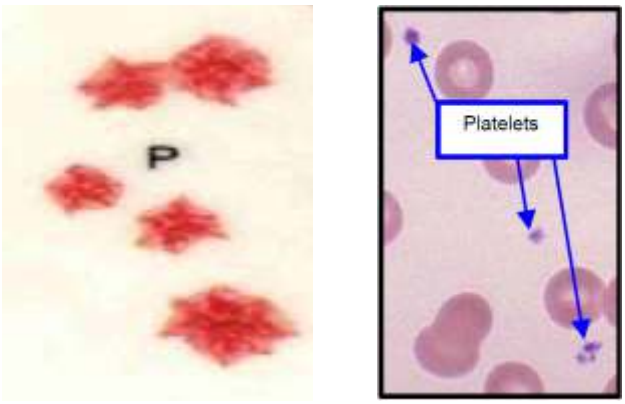


Fig 6. 6 Platelets

HAEMOGLOBIN ESTIMATION

1. Haemiglobincyanide (Cyanmethaemoglobin) Method

The Haemiglobincyanide (Cyanmethaemoglobin) method is the internationally recommended method for determining the hemoglobin concentration of blood.

Principle

Blood is diluted in Drabkin's solution, a solution containing potassium cyanide and potassium ferricyanide. Potassium ferricyanide oxidizes hemoglobin to methaemoglobin. Methaemoglobin then combines with potassium cyanide to form Cyanmethaemoglobin (HiCN). The absorbance of the solution is measured in a spectrophotometer at a wave length of 540 nm against Drabkin's solution as a blank. The result is calculated using the formula provided below and it is expressed in gm/dl.

Test Sample

Venous or capillary blood collected into an EDTA tube. Alternatively, free flowing capillary blood may be added directly to the diluting fluid and measured.

Procedure

1. Pipette 4ml of Drabkin's solution into a test tube.
2. Pipette 20 µl (0.02 ml) of properly mixed blood.
3. Clean outside of pipette and wash out the blood in the tube containing the diluent.
4. Mix and leave for 5-10 minutes for the reaction to complete.
5. Using drabkin's solution as a blank, read the absorbance in the spectrophotometer at wavelength 540 nm.

Calculation

Use the following formula: Hb in g/dl $= \frac{A \times 64500 \times DIL}{44 \times D \times 1000 \times 10} = A \times 29.3$

Where:
- A = Reading of absorbance of Hb solution
- 64500 = Molecular wt. of Hb
- 44 = Millimolar extinction coefficient
- D = Thickness of cuvette
- 1000 = Conversion factor of mg to gm
- DIL = Dilution Factor = 200

Notes on Technique:

- The blood sample must be properly mixed before sampling and allowed to warm
- Care should be taken when handling potassium cyanide.
- Use clean tubes and pipettes.

Comments

The Cyanmethaemoglobin method is the reference method for Hb estimation because: a) all Hb forms except sulphaemoglobin are estimated, b) highly reliable and stable reagents are available and c) the method can be easily standardized.

Normal Ranges

Adult males: 13 – 17 g/dl
Adult females: 11.5 – 16.5 g/dl
Newborns and infants: 14 – 22 g/dl

2. Oxyhaemoglobin Method

The HbO2 method is the simplest and quickest method for general use with a photometer. Its disadvantage is that it is not possible to prepare a stable HbO2 standard, so the calibration of these instruments should be checked regularly using HiCN reference.

Direct Reading Portable Haemoglobinometers

Color Comparators

These are simple clinical devices that compare the color of blood against a range of colors representing hemoglobin concentrations. They are intended for anemia screening in the absence of laboratory facilities.

Portable Hemoglobinometers

Portable hemoglobinometers have a built-in filter and a scale calibrated for direct reading of hemoglobin in g/dl or g/l. They are generally based on the HbO2 method. Some hemoglobinometers require dilutions of blood and others do not require dilutions because blood is drawn into cuvettes containing certain chemicals.

Noninvasive Screening Tests

Methods are being developed for using infrared spectroscopy at body sites, mainly a finger, to identify the spectral pattern of hemoglobin in an underlying blood vessel and derive a measurement of Haemoglobin concentration.

3. Automated Haemoglobin measurement

Most automated counters measure Haemoglobin by a modification of the manual HiCN method with cyanide reagent or with a nonhazardous chemical such as sodium lauryl sulphate, which avoids possible environmental hazards from disposal of large volumes of cyanide containing waste. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A nonionic detergent is included to ensure rapid cell lysis and to reduce turbidity caused by cell membranes and plasma lipids. Measurements of absorbance are made at a set time interval after mixing of blood and the active reagents, but before the reaction are complete.

Total White Blood Cell Count

White cell count is an important component of the blood count. White cells can be counted either manually or automatically. Anticoagulated venous blood is added to a diluent at a specific volume. The diluent lyses the erythrocytes, but preserves leukocytes. The diluted blood is added to the hematocytometer chamber. The diluent used is 2% acetic acid +gentian violet.

Manual Method

1. Pipette 0.95 ml of the diluents in a 75x10 mm tube.
2. Add 0.05 ml (50 µl) of blood to the tube.
3. Tightly seal the tube and mix the contents for one min.

4. Fill the counting chamber by means of a capillary tube.
5. Count the cells in the four "white cell" corner squares.

Note: Each one of the four corner squares "W" has an area of 1mm^2. These large squares contain 16 smaller secondary squares, each with an area of 0.04 mm^2

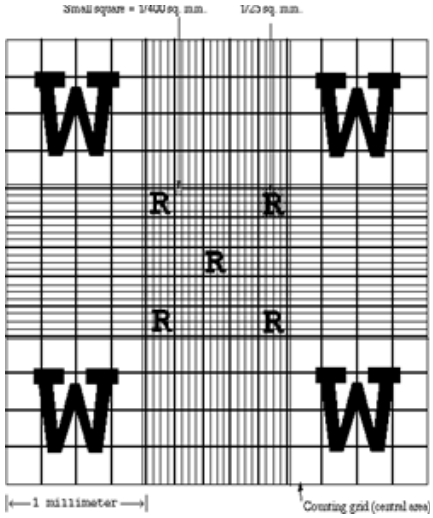


Figure 51: Hematocytometer. The areas indicated by "W" are used for counting white blood cells.

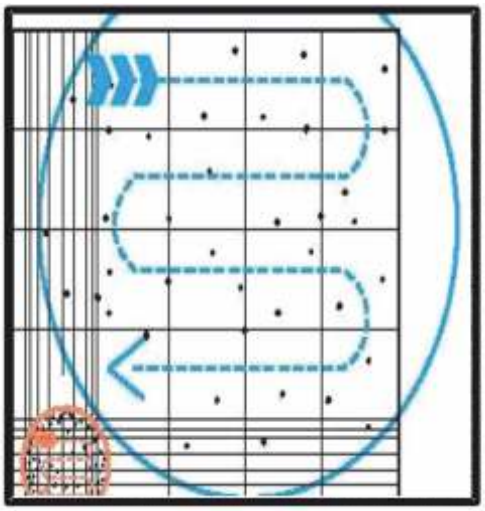


Figure52:Illustration demonstrating the direction of counting WBCs on

Calculation

Dilution

$$\frac{\text{50 µl blood}}{50} = 1 \text{ volume of blood}$$

$$\frac{\text{0.95 ml (950 µdiluent)}}{50} = 19$$

$$\frac{1}{19+1} = \frac{1}{20}$$

Dilution factor = 20

Number of WBC in Whole Blood

$$\text{Count} = \frac{\text{No.of cells counted} \times \textit{dilfactor}}{\text{volume}}$$

= N 0.1

$$\frac{\text{0.95 ml (950 µdiluent)}}{50} = 19$$

$$\frac{1}{19+1} = \frac{1}{20}$$

Dilution factor = 20

Number of WBC in Whole Blood

$$\text{Count} = \frac{\text{No.of cells counted}}{\text{volume}} \times \textit{dilfactor}$$

= N 0.1× *dilution factor*

$= N \times 10 \times 20 = N \times 200$

Normal Range

The normal range is: $4 - 10 \times 10^9/l$

Reticulocyte Count

Reticulocytes are immature red blood cells which contain remnants of ribosomal RNA. They are normally present in the bone marrow and blood stream. The number of reticulocytes in peripheral blood is a reflection of erythropoietic activity.

Principle

Given their acidic nature, ribosomes of reticulocytes react with certain alkaline dyes such as brilliant cresyl blue or new methylene blue to form dark blue/purple granules or filaments. This reaction takes place only in vitally-stained unfixed preparations. If, however, blood films are dried, fixed and then stained with basophilic dyes, reticulocytes appear as diffused basophilic RBCs (polychromatic RBCs). Since reticulocytes ripen within about 24 hours in blood circulation, the reticulocyte count must be done on fresh blood.

Method

1. Add 2-3 drops of reticulocyte dye in a tube.
2. Add 2 drops of blood and mix.
3. Incubate at 37°C for 15-20 min.
4. Re-suspend by gentle mixing and prepare a film on s glass slide. Leave to dry.
5. Examine under microscope using oil immersion lens.
6. Select a well prepared, well stained area. Reticulocytes appear as pale greenish cells with dark blue granules or filaments.

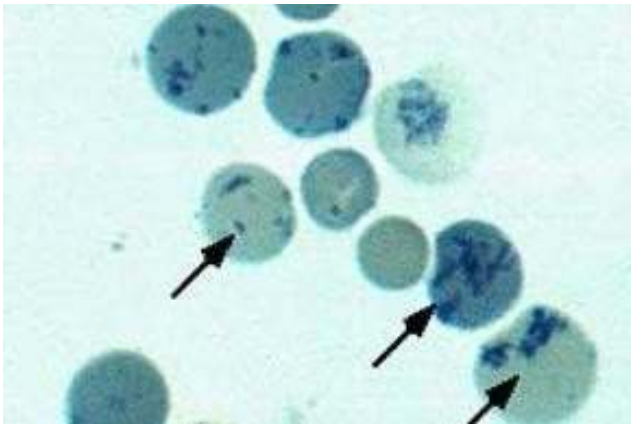


Figure 49: Reticulocytes in peripheral blood. They appear as greenish cells with blue filaments

7. Count the number of reticulocytes in 40 successive fields.
8. Count the number of total RBCs in the fields: 1, 10, 20 and 30.
9. Determine the % ratio of reticulocytes to total RBCs.

Calculation

Number of reticulocytes in 40 fields = X

Number of total RBCs in four fields = Y

Reticulocyte count = $\frac{X}{N \times Y} \times 100\%$

Normal Ranges

- Adults: 0.5 -2.5 %
- Infants: 2-5 %

Red Cell Indices

The calculation of the size and haemoglobin content of the red cells from the Hb, PCV and red cell count, have been widely used in the classification of anemia. The three most common indices are the MCV, the MHC and the MCHC.

Mean Corpuscular Volume (MCV)

The MCV is the average volume of a single red cell expressed in femtoliters (fl) or 10-15 L. It helps in determining the size of the RBC. The PCV and red cell volume are used in its calculation.

Calculation Formula

$$MCV = \frac{PCV\ \% \times 10}{\text{RBC count (the number of millions not the actual count)}}$$

Example:

PCV = 0.45 L/L (45%), RBC = 5 x 1012/L

$$MCV = \frac{PCV \times 10}{RBC} = \frac{45(\%)}{5} \times 10 = 90\ fl$$

Reference Range

The MCV reference range is 83-101 fl. The MCV is increased in macrocytic anemias (e.g. megaloblastic anemia) and decreased in microcytic anemias (e.g. iron deficiency, thalassemia)

MCV value is within the normal range → Normocytic.
MCV value is above the normal range → Macrocytic.
MCV value is below the normal range → Microcytic.

Mean Cell Hemoglobin (MCH)

MCH is the average weight in picograns of Hb in one red cell. The Hb level and the RBC count are used for calculation.

Calculation Formula

$$MCH = \frac{Hb\ (g/dl) \times 10}{\text{RBC (use the number of millions rather than the actual count)}}$$

Example:

Hb = 150g/l (15 g/dl), RBC = 5 x 1012 /L

$$MCH = \frac{15(g/dl) \times 10}{5} = 30\ pg$$

Reference Range

The MHC reference range is 27-32 pg. MHC is increases in macrocytic anemia and decreased in microcytic anemias.

MCH value iswithin the normal range → Normochromic
MCH value is above the normal range → Hyperchromic
MCH value is below the normal range → Hypochromic

Mean Cell Hemoglobin Concentration (MCHC)

The MCHC is the concentration of haemoglobin per unit volume of red blood cells expressed as a percentage, g/dl or g/l. Hemoglobin and PCV are required to calculate MCHC.

Calculation Formula

$$MCHC = \frac{Hb(g/dl) \times 100}{PCV\%}$$

Example:

If one liter of blood contains 0.45 liters of packed cells and 150g of Hb (150g Hb are contained in 0.45 liters of RBCs). What is the Hb concentration?

Hb = 15 g/dl (150 g/l), **PCV** = 45% (0.45 l/l)

$$\text{MCHC} = \frac{\text{Hb (g/dl)} \times 100}{33.3\ \text{g/dl}} = \frac{15 \times 100}{333.3\ \text{g/l PCV\%}} = \frac{}{45} = 33.3\%\ \text{or}$$

Reference Range

The reference range for MCHC is 32-36 g/dl or (320-360 g/l).

A fully saturated red cell has a hemoglobin concentration of 36 g/dl. MCHC is a useful guide to the degree of hypochromia present in iron deficiency anemia. The Hb and PCV can be estimated reasonably accurately and the derived MCHC is therefore a reliable parameter.

Automated Estimation of Red Cell Indices

In automated counters, MCV is measured directly, but in semiautomated counters MCV is calculated by dividing the PCV by RBC. MCH is derived from the Hb divided by RBC. The MCHC is derived from the Hb and the PCV with instruments that measure the PCV and calculate the MCV, whereas when the MCV is measured directly and the PCV is calculated, the MCHC is derived from the Hb, PCV and RBC

CHAPTER - 7

DETERMINING HAEMOGLOBIN AND HAEMATOCRIT

7.1 DETERMINING OF HAEMOGLOBIN LEVEL (USING UNDILUTED BLOOD)

Haemoglobin (Hb) is the part of the red blood cell that carries oxygen from the lungs to all cells of the body. If haemoglobin is low (lacking in our body), there will not be enough Hb to carry oxygen to the tissues. When this happens, the patient is said to be anaemic.

Principle of the method:

Blood obtained by pricking the finger, is run directly into a special slide.
The color is compared with a series of colored glasses in a Lovibond comparator.

Materials needed:

* Lovibond reader.
* Disc A covers the number 20, 24, 28, 32, 36, 40, 46, 52, 58.
* Disc B covers the number 64, 70, 76, 84, 92, 100, 110, 120.
* Special 0.004 slide.
* Gloves / Blood lancets / Cotton.
* Savlon / water / tissue paper / dustbin

Method:

1. Clean the 2 slides thoroughly, first with water and then with savlon; rinse Well, and dry with clean tissue paper. If the cleaning is not correctly done, air bubbles form when the blood is run into the space between the 2 slides, leading to incorrect result.
2. Place the 2 slides in opposition and clip them together (figure 9. 1 A and B).
3. Fill the space between the 2 slides, from the side, with capillary blood (figure 9. 1C)
4. Remove the clip, as the 2 slides will stick together by capillarity.
5. Place the slides carefully into the right-hand holder of the comparator.

   The blood film must completely fill the right-hand field of view and be free from air bubbles.
6. Hold the comparator with your arm straight and face a white light (direct sunlight or incorrect artificial light gives incorrect result).
7. Move the disc until the glass color corresponds to the color of the blood. The reading should be done AS SOON AS POSSIBLE after filling the cell (within one minute).

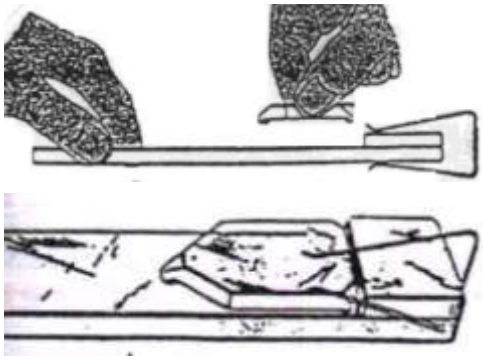


Fig 9.1 (A, B)

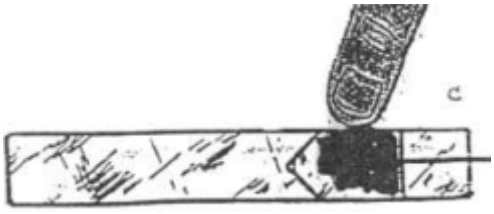


Fig 9.1 (C)

8. Use the conversion table to translate the number written on the disc in g/100 ml.

9. Wash the 2 slides as soon as possible after use (as in step 1).

Attention:

Please handle the glass slides with good care, as their cost is quite expensive.

CONVERSION TABLE

Reading on disc A or B	g /100ml
20	3.3
24	4.0
	4.7
32	5.3
36	6.0
40	6.7
46	7.3
52	8.7
58	9.7
64	10.7
70	11.7
76	12.7
84	14.7
92	15.3
100	16.7
110	18.3
120	20.0
130	21.7

7.2 DETERMINING OF HAEMATOCRIT

Haematocrit (Hct) is the proportion of packed red cells volume per whole blood volume and is presented in percentage as follows:

$$\text{Haematocrit} = \frac{\text{total volume of packed red cell x 100}}{\text{Volume of whole blood}}$$

Example: 10 ml of one patient's whole blood has 4ml of packed RBC, so % Hct of this patient is:

$$\frac{4}{10} \times 100 = 40\%$$

The haematocrit indicates the proportion volume of RBC in order to know if the patient has anaemia.

Haematocrit measurement

Principle of the method:

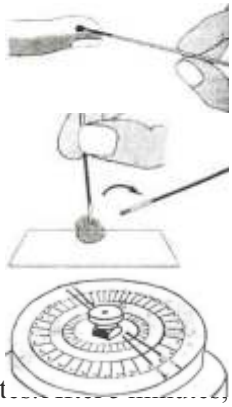
Blood is collected into a small capillary tube and is spun at high speed. The blood will be separated

firmly into 3 parts: upper part is plasma, middle part is a small volume of white blood cells and the lower is packed red cells. The fraction of packed red cells volume to the entire blood volume is measured as the percent haematocrit.

Materials needed:

Heparinised capillary tube: It contains dried heparin as anticoagulant inside so the blood will not clot.
Clay sealer
Microhaematocrit centrifuge (electricity needed)
Specially designed scale for reading the result.
Rubbing alcohol or savlon / Gloves / Blood lancets / Cotton / Container for disposal

Method:



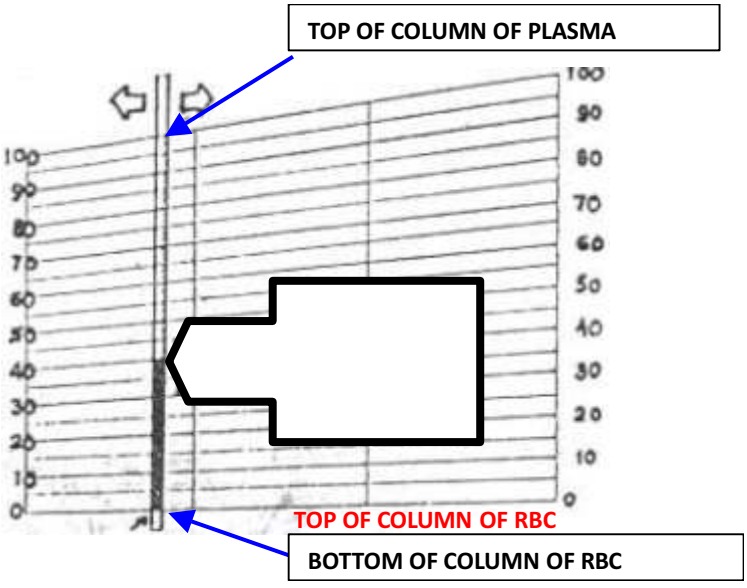
1. Collect blood by using blood lancet as the safety method. Fill 3/4 of the capillary tube by applying the blood to the side opposite to the red coloured end.
2. Plug the red coded end of the tube into the clay completely (depth of about 2 mm).
3. Place the tube in the numbered slots in centrifuge head. The sealed end of the tube should point outward, away from the center. Don't forget to balance the tubes.
4. Centrifuge at high speed (10,000 to 12,000 rounds per minutes) for 5 minut[illegible] allow the spinner head to stop.
5. The tube shows 3 layers of blood.

   - At the top, a column of plasma (P)
   - In the middle, a very thin layer of WBCs (WC)
   - At the bottom, a column of RBCs (RC)

   The packed RBC volume reading is made exactly at the top of the RBC column.

6. Read the layers by using the scales:

   - Hold the tube against the scale.
   - The bottom of RBC column (not the bottom of the tube) should be aligned with the horizontal zero line.
   - Move the tube across the scale until the top of the plasma column reaches 100% mark line.
   - Make sure that the bottom of RBC column is still on the line O (zero) and the tube is always vertical.



7. The line that passed through the top of the RBC column gives packed red cell volume fraction (40% in the figure above).

Normal values	Haematocrit
Men (>15 years old)	40-50%
Women (>15 years old)	37-43%
Children (>2-15 years 0ld)	38-44%
Baby infants (10 days old -2 year old)	35-49%
Newborn infants (1 day old-9 days old)	44-64%

Lower values of % Hct are found in patients suffering from anaemia. For example: lower than 40% in men and lower than 37% in women.

High values of % Hct are found in cases where the patient is suffering from a loss of plasma: severe burns, dehydration, infant diarrhea, dengue hemorrhagic fever, etc.

A scale for the haematocrit measurement

Microhaematocrit centrifuge

Erythrocyte Sedimentation Rate (ESR)

If anticoagulated blood is allowed to stand undisturbed in a vertical tube, the red cells will gradually settle to the bottom of the tube leaving a clear layer of plasma.

Red blood cells possess a negative charge and when suspended in normal plasma, rouleaux formation is minimal and the sedimentation of the cells is slow. Changes in the proportion and concentration of plasma proteins, particularly macro-molecules like fibrinogen and globulin, reduce the negative charge and thus increase the rate of rouleaux formation. This in turn increases the rate of sedimentation.

The ESR test is a non-specific test that indicates changes in plasma protein concentrations due to infection or injury.

Definition

The erythrocyte sedimentation rate (ESR) is the rate at which the red blood cells settle in a tube over a given amount of time.

Factors Affecting ESR

Plasma Protein

Changes in plasma protein occur rapidly following tissue injury or in response to inflammation. Increased concentration of fibrinogen and immunoglobulins will increase roulaux formation and hence the rate of sedimentation. Plasma albumin retards sedimentation of RBCs.

RBC Size and Number

The size and number of RBCs that show alterations in their biconcavity, such as spherocytes and sickle cells, usually do not increase the ESR rate, unless there is severe anemia. Increased cell mass will decrease the sedimentation rate (e.g. polycythemia).

Technical Factors

- Tilted ESR tube. ESR tubes should always be in a perpendicular (straight up) position. Slight deviations from the absolute vertical can increase results.
- A temperature higher than room temperature (18- 25 º C) accelerates sedimentation. Therefore, ESR tubes should not be exposed to direct sunlight or anything that may alter their temperature. If the test is to be carried out at a higher temperature, a normal range should be established for that temperature.
- Vibration can reduce the rate of erythrocyte sedimentation.

Methods of Measuring ESR

There are two methods for ESR measurement: The Westergren method and the Wintrobe method. The recommended method is the Westergren Method.

Westergren Method

Principle

The test measures the sedimentation of red cells (in diluted blood) after one hour in an open-ended tube mounted vertically on a stand. The recommended tube is a straight glass or rigid plastic transparent tube 30 cm in length and not less than 2.55 mm in diameter. The bore must be uniform to within 5% throughout (0.05 mm).

Procedure

1. Venous blood is collected into a sodium citrate tube, or collected into an EDTA tube and diluted accurately in a proportion of 1 volume of citrate to 4 volumes of blood. The usual practice is to collect blood directly into the sodium citrate tube.
2. Mix the blood sample thoroughly and then draw it up into the Westegren tube to the 200 mm mark by means of a mechanical suction device.
3. Place the tube exactly vertical and leave undisturbed for exactly 60 minutes, free from vibrations and not exposed to direct sunlight
4. After the 60 min are over, read to the nearest 1 mm the height of the clear plasma above the upper limit of the column of sedimenting cells.
5. The result is expressed as ESR= X mm in 1 h.

Notes on procedure:

- The test should be carried out within 4 hours of collecting the blood. A delay up to 6 hours is permissible provided that the blood is kept at 4◦C.
- EDTA blood can be used 24 hours on a specimen that was kept in 4◦C if 1 volume of 109 mmol/l trisodium citrate is added to 4 volumes of blood immediately before the test is peformed.

ESR Normal Ranges

Men

- Ages 17-70: 10 – 14 mm/hour
- Ages > 70: about 30 mm/hour

Women

- Ages 17-70: 12-20 mm/hour
- Ages >70: about 35 mm/hour

Pregnant Women:

- First half: 48 mm/hour (62 if anemic)
- Second half: 70 mm/hour (95 if anemic)

General Applications of ESR

Conditions that Cause a High ESR

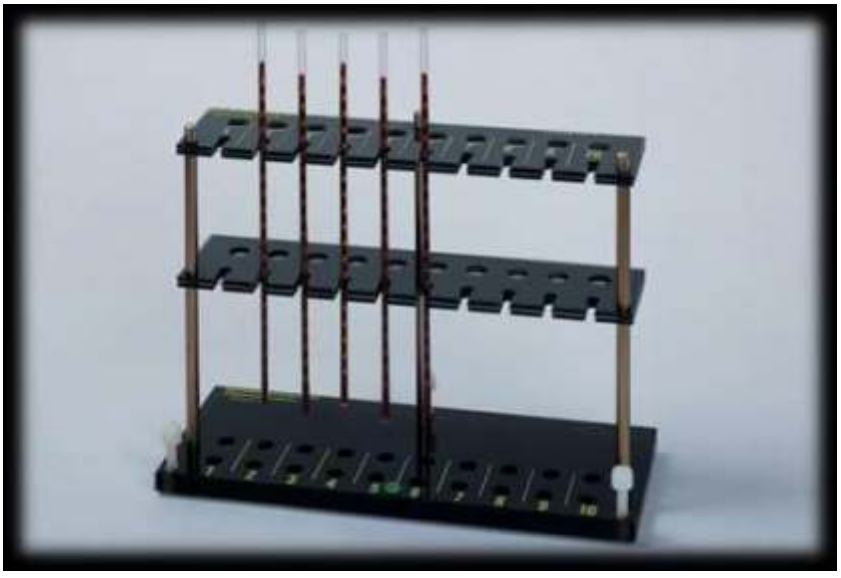
- ***Malignancy:***
  - Malignant lymphoma
  - Breast and colon carcinomas
- ***Hematologic:***
  - Macrocytosis.
  - Anemia of acute or chronic diseases
- ***Inflammatory Disorders:***
  - Rheumatoid arthritis
- ***Infections:***
  - Tuberculosis
  - Acute hepatitis

Conditions that Cause a Low ESR

- Polycythemia
- Sickle cell anemia
- Hypofibrinogenemia
- Congestive Heart failure
- Leukocytosis

Note:

Other factors that influence ESR include: age, sex, menstrual cycle and the use of certain drugs.



CHAPTER - 8

BLOOD TRANSFUSION TESTING

A blood transfusion is the taking of a small amount of blood from a donor and giving it to a patient. Transfusion testing is the group of tests we do on a donor when a patient is in need of a blood transfusion.

These tests include:
- Haemoglobin or Haematocrit
- Blood group
- Malaria smear
- Hepatitis B test
- HIV test

Transfusion is done only when the patient present low haemoglobin or low haematocrit and the medic decide that it will be life threatening unless the patient receives blood transfusion.

Transfusion is serious and can be dangerous. A patient receiving the wrong blood group can become severely ill or even die. A patient receiving blood positive for malaria, Hepatitis B or HIV will develop these diseases.

It is important to follow the blood transfusion chart closely when a transfusion is required.

REGISTRATION:

All tests performed for transfusion are registered on a blood transfusion request card and in a blood transfusion register book.

The following information should be included:

Patient information:	Donor information:
Date	Date
Name	Number
Age	Age
Sex	Sex
Blood group	Haemoglobin or haematocrit
Haemoglobin or haematocrit	Malaria smear result
Diagnosis	Agreement of transfusion (Yes or No)

**** Note that the donor's name does not appear on the register.***

CONFIDENTIALITY

Donor confidentiality is very important!!!

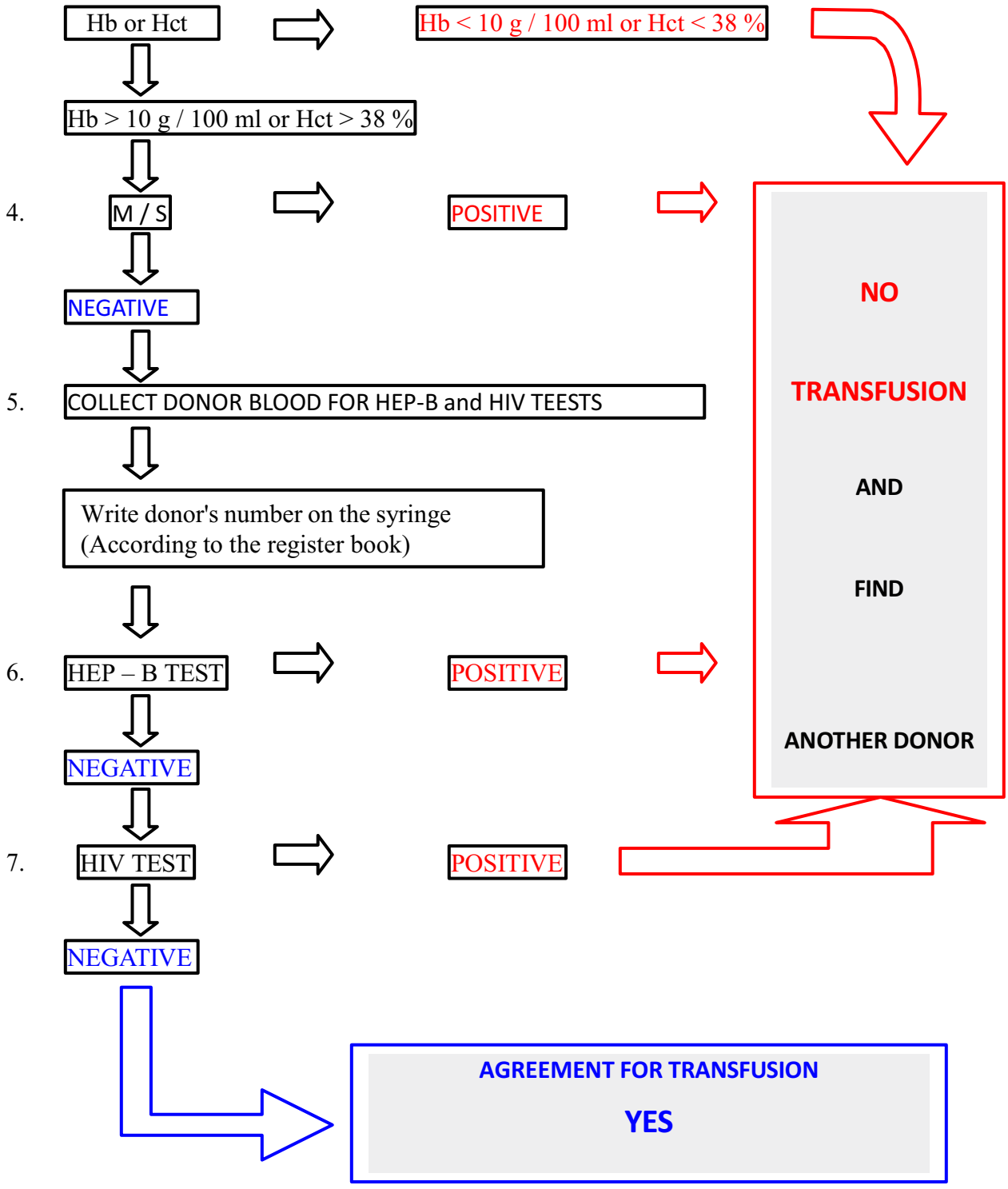
Because of the social problems attached to the diagnosis of HIV it is unfair to disclose information to anyone. The tests used are very sensitive: there are no false negative results. Therefore they are suitable for blood donor testing because we are sure of a negative result and that someone's blood is safe for donation. On the other hand, the tests used are poorly specific: false positive results are common. Therefore these tests are not suitable for diagnosis and a positive result always needs to be confirmed by a more specific test.

NEVER ALLOW ANYONE INTO THE LAB WHILE MAKING THE HEP-B OR HIV TESTS. NEVER DISCUSS OR RECORD THE RESULT OF THESE TESTS EVEN WITH THE MEDIC!!!

** The medic or nurse must never ask for the Hep-B or HIV result.

PROCESS OF BLOOD TRANSFUSION TEST:

1. Fill information of the patient and donor in the register book and blood transfusion request card.
2. Test for blood group of the patient and donor.
3. If donor has the same blood group as the patient, test for Hb or Hct.



BLOOD GROUP:

Before a blood transfusion you have to check the patient's blood group and the donor's blood group to know if they are compatible.

What is a blood group?

It is a chemical substance on the membrane of a red blood cell, which is call ANTIGEN. Each person has his own type of antigen since they were born. Blood is divided into 4 groups by the presence of its antigen and we call them as group A, B AB and O. Each blood group antigen has a different character.

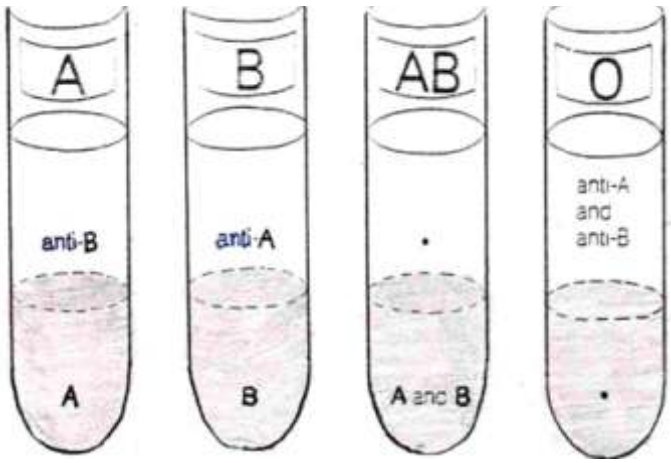
In the plasma, there are some substances called ANTIBODIES, which act as the protectors of your body. They attack and kill bacteria, virus and any antigen, which does not belong to your body.

Each blood group antigen has a corresponding antibody. Antibodies are specific for each antigen. You are born with the antibody for the group you don't have

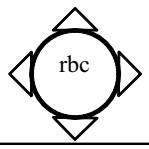
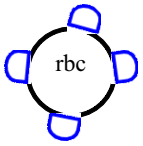
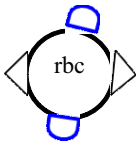
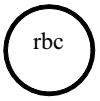
PATIENT'S BLOOD GROUP

ANTIBODIES
In the plasma

ANTIGENS
On the red blood cells



The following drawings in this table propose the idea of each group antigen and antibody.

Blood Group	A	B	AB	O
Antigens	A= 	B = 	AB = 	No antigen 
Antibodies	Anti - B 	Anti - A 	No antibodies	Anti – A and Anti B 

This is why you cannot just give anybody's blood without checking the group before transfusion.

If blood group A patient received blood group B from the donor, anti B in the patient's blood will attack RBCs from donor because they have antigen B on. This fighting reaction will cause the patient to die quickly. You don't need to always give the exact same blood group to a patient as long as you give the group that the patient doesn't have the antibodies for.

However, always try to transfuse donor blood with the same group as the patient's group.

		Donor Group			
		A	B	AB	O
Patient's Group	A	OK	X	X	OK
	B	X	OK	X	OK
	AB	OK	OK	OK	OK
	O	X	X	X	OK

LABORATORY EXAMINATION FOR BLOOD GROUPING

To know the blood group of a person, we test their red blood cells for the antigen. The test can be done by using anti – A and anti – B sera.

Principle:

A sample of blood from the donor or patient will be mixed with the known antibodies and the clumping of its reaction will be observed. We call this reaction "agglutination". The agglutination seen means that the antigen and the antibody has corresponded and stuck together.

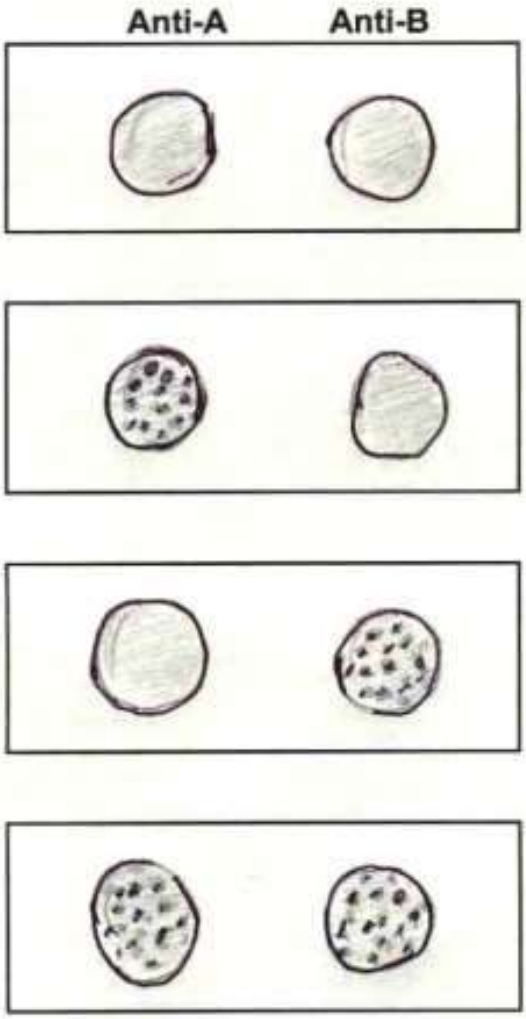
Reaction with Anti - A	Reaction with Anti - B	Blood Group
+	–	A
–	+	B
+	+	AB
–	–	O

Materials:

- Gloves, blood lancet, savlon or rubbing alcohol, cotton
- 2 microscopic slides
- Antiserum-A and antiserum-B
- Disinfectant solution

Method:

1. Put the gloves on.
2. Check the antiserum whether it has expired or not. If it has expired, check for a new bottle in the fridge. Check that the antiserum solution is not cloudy. If so, it has gone bad (wipe condensation away first), check for a new bottle.
3. Collect blood specimen from the finger as described in lesson 7.2.
4. Handle a clean slide only by the edges.
5. Place two drops of blood on the slide at least 2 cm apart.
6. Carefully and **without touching the end of the dropper to the blood**, add one drop of antiserum A to one drop of blood and add one drop of antiserum B to the other drop of blood.
7. With 2 different corners of the other slide, mix each drop of blood with the added antiserum separately.
8. Pick the slide up and gently rock back and forth looking for clumping RBC (agglutination) for 2 minutes
9. Read the result as follows:


Anti-A
Anti-B
Blood Group O
Blood Group A
Blood Group B
Blood Group AB

CHAPTER - 9

MALARIA

Malaria is a disease caused by a parasite, *Plasmodium*, which infects red blood cells. Malaria is spread by **mosquitoes**. Mosquitoes suck up the malaria parasite from the blood of an infected person and transfer parasites to another person when biting them.

There are 4 kinds of Plasmodium:

- *Plasmodium falciparum* causes the severe malaria, which can cause coma and lead to death.
- *Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale* give mild malaria cases.

Plasmodium falciparum more common in tribal areas and tarai areas and *Plasmodium vivax* are often found and *Plasmodium malariae* is seldom found whereas *Plasmodium ovale* is rarely found.

Symptoms and clinical signs:

A patient with malaria will complain of fever, chills, headache, and joint pain. During the clinical exam, the medic or doctor sometimes finds anaemia and big spleen (especially among children). In cases of severe malaria, the patient may also be unconscious.

Diagnosis:

Microscopic examination of blood will show parasites inside the red blood cells. The name of the laboratory exam is MS or M/S (malaria smear).

- Plasmodium falciparum or PF are small and ring shaped. They are often found in great number in 4+ or ++++ cases, more than 4 % of RBCs are infected.
- Plasmodium vivax or PV and Plasmodium ovale or PO are bigger than PF, but usually are less numerous and less harmful.
- Plasmodium malariae or PM are also bigger than PF, found in very small quantities in the blood and they develop very slowly.

Treatment:

- In this area, usually patients infected with PV, PO or PM are treated with Chloroquine.
- For patients with PF, the treatment might be done by Quinine, Mefloquine, Artesunate and their combinations with other medicines (As per Malaria Tt Protocol).

Prevention:

Malaria is spread by a mosquito. This mosquito is called Anopheles and it usually bites from sunset until dawn. To prevent malaria, it is necessary to avoid being bitten by the Anopheles mosquito. This can be done by:

- Covering up between sunset and dawn.
- Taking a bath before sunset or after dawn then use repellent.
- Sleeping under a mosquito bed net.
- Killing mosquitoes, reducing mosquito-breeding places.

To prevent severe malaria from developing, have your blood checked for every fever.

9.1 MALARIA LIFE CYCLE

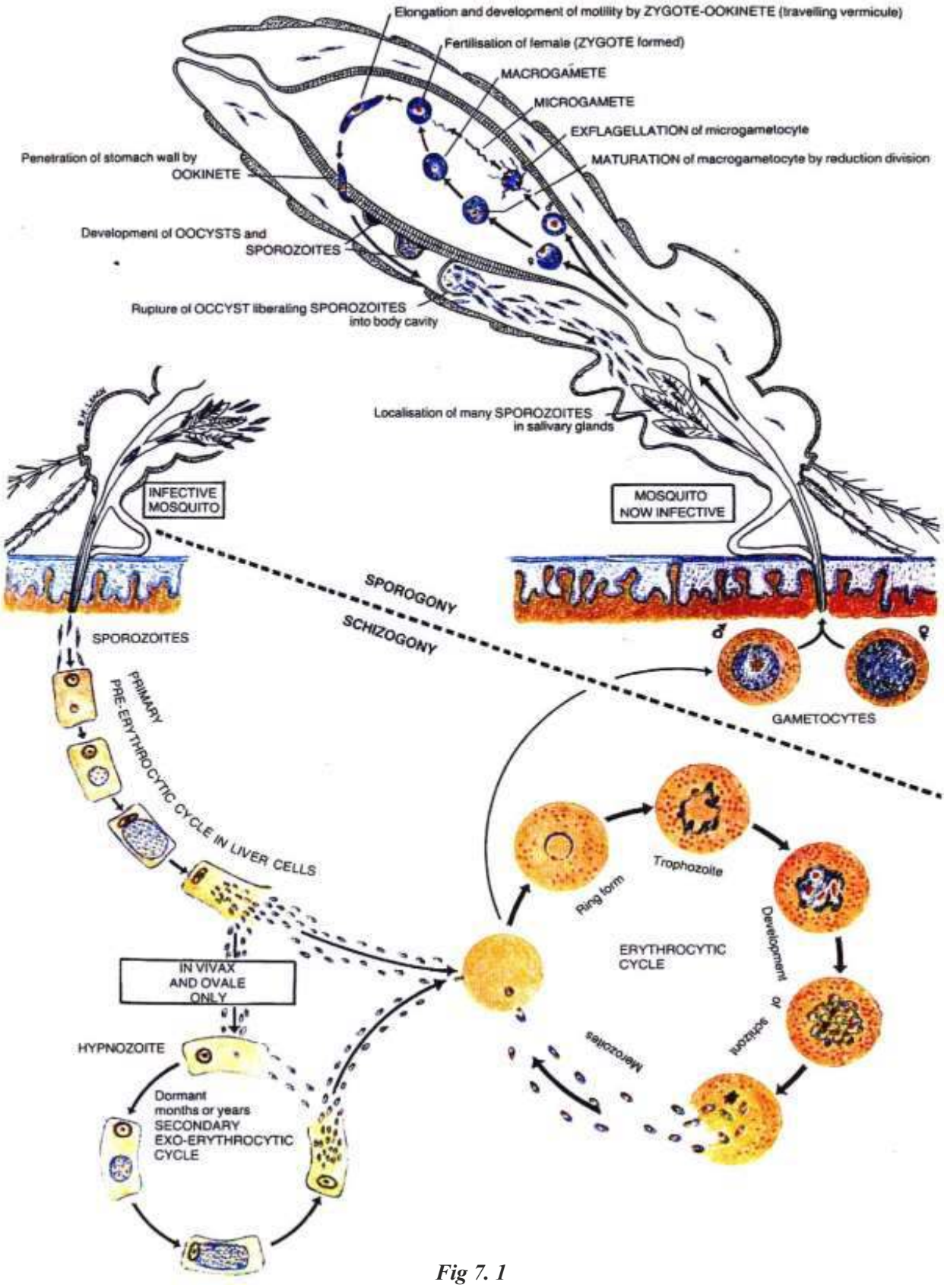


Fig 7. 1

Female anopheles mosquito needs to eat blood when it is pregnant. The anopheles infected with Plasmodium bites a person to suck his blood and at the same time, it releases SPOROZOITES in the blood of this person. SPOROZOITES are the infective form of Plasmodium (see Fig.7. 1)

Pre – Erythrocytic Cycle in Liver cells (Cycle in liver before RBC cycle):

1. After sporozoites were released to the blood stream, they reach the liver and enter the liver cells then develop. Each parasite grows and divides up to 30.000 new parasites, remaining in each liver cell, called hepatic schizont. Then liver cells burst, and release a great number of new parasites, called MEROZOITES into the blood stream.

   For *Plasmodium vivax* and *Plasmodium ovale*, some parasites remain in liver cells, as sleeping forms (hypnozoites). In the liver cells, these sleeping forms cannot be destroyed by Chloroquine. They can wake up and produce new merozoites into blood stream again causing a new malaria episode, not caused by a new infection. This is called relapse, which may occur from time to time after initial infection, during 2 – 3 years.

Erythrocytic Cycle (Cycle in Red blood Cell):

2. After each merozoite was released into blood stream, it enters a RBC and develops. The merozoites that cannot enter a RBC within 5 minutes are destroyed. Once inside the RBC, merozoite becomes TROPHOZOITE (PVT = Plasmodium Vivax Trophozoite).

3. The Trophozoite grows and divides itself. It becomes a SCHIZONT: each schizont can contain up to 32 new parasites depending on the type of Plasmodium, which are also called merozoites. Then the infected RBCs burst and release the new merozoites in the blood. This provokes the peak of fever.

DESTRUCTION OF RBCs	cause	ANAEMIA
DEAD RBCs ELIMINATED BY SPLEEN	cause	BIG SPLEEN

   Development of the parasite in the RBCs takes 42 – 48 hours for PV and 36 – 48 hours for PF. Delay between infected mosquito bite and the first peak of fever is called **"incubation period"** and it takes about 2 weeks (12 – 17 days for PV and 9 –14 days for PF).

4. Each new merozoite will infect one new RBC and make a new cycle, causing the fever to go UP and DOWN. Chloroquine destroys schizonts, so it stops the cycle in the blood (but not in the liver: see 1. above).

5. After some cycles in the RBCs, instead of developing into schizonts, some merozoites develop in a different way. They become sexual reproductive form called GAMETOCYTES. There are male gametocytes and female gametocytes. Their presence in the blood of an infected person does not cause any disease and if a mosquito does not take them up, they die.

Infective form in the mosquito:

6. When the anopheles mosquito bites and sucks an infected person's blood, in the mosquito's stomach, all the trophozoites and schizonts die. Only gametocytes can survive. Fertilization occurs between male and female Gametocytes and produces a ZYGOTE (egg). The zygote enters the stomach wall of the mosquito, and develops there. Each egg gives birth to a great number of new sporozoites, which is the infective form of malaria. Sporozoites then collect in the salivary glands of the mosquito.

   Life cycle of each type of Plasmodium malaria are different in the number of merozoites contained in mature schizonts, the incubation period, the length of time of the parasite cycle in the RBCs and the presentation of relapse.

Characteristics	*P. Falciparum*	*P. Vivax*	*P. Malariae*	*P. Ovale*
No. of merozoites in Each mature liver Schizont	Up to 30,000	10,000	15,000	15,000
No. of merozoites in each mature RBC schizont	8 – 32 Rarely seen in Peripheral blood	Up to 24	10 - 12	8 - 10
A cycle duration of parasite in RBC	36 – 48 hr.	42 – 48 hrs	72 hrs	48 - 50 hrs
Incubation period	9 – 14 days Exceptionally up To 1 year	12 – 17 days	18 – 40 days	16 – 18 days
Time of relapse after initial infection	No relapse	8 – 10 weeks, or 30 – 40 weeks, or Up to 8 years	Up to 53 years	No certain report
Entering of merozoites to RBCs	Enter every stage Of RBCs	Particularly enter Young RBCs	Usually invade Older RBCs	Usually infect Younger RBCs

RESISTANCE

The appropriate treatment of malaria in each area depends on the susceptibility of malaria parasite to the anti malarial drugs. Presumptive treatment, wrong lab diagnosis, and the incorrect use of these drugs (e.g. self treatment) contribute to the parasites resistance, which means that the parasite can survive and/or multiply despite taking medicine.

The first anti-malarial drugs were discovered about 3000 years ago, but since 1950 resistance appeared among P. falciparum against Chloroquine and this parasite continues a great facility to become resistant to many other malaria drugs. The falciparum parasites on the Thai – Myanmar border are the most multidrug resistant in the world.

The follow up of patient is very important in order to monitor the efficacy of the drugs.

9.2 MALARIA SMEAR

It is a laboratory exam that finds out if a patient has malaria. The examination is done with blood, because malaria parasites infect Red Blood Cells.

Method

1) On a tray, prepare:

- Clean slides
- Cotton
- Savlon or rubbing alcohol
- Gloves
- Blood lancets
- Little dustbin for dirty cotton
- Glass container for used blood lancets
- Pencil

2) Clean one of the slides, holding it by the edges:
!! Never touch the slide surface !!

Fig 7. 2

3) Put on the gloves.

4) Clean the third or the fourth finger of the patient with cotton soaked in rubbing alcohol or savlon (see figure 7. 2)

In baby under 6 months or very small children, it might be difficult and also dangerous to get the blood from the finger as the lancet may pierce finger bone and cause possible infections. You may try to take blood from the side of the heel (as shown by blue arrows in figure 7. 3) or from the side of big toe instead (as shown by red arrow in figure 7. 3)

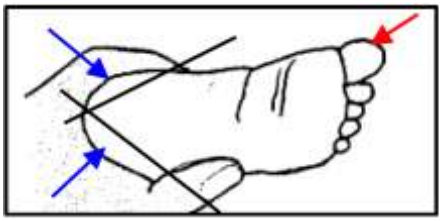


Fig 7. 3

5) Open the blood lancet paper, from the side opposite to the sharp end.

6) Remove the lancet from the paper and prick the finger or the site firmly and rapidly (see figure 7. 4).
!! Place the used lancet in the glass container or dispose of it properly!!

Fig 7. 4

7) With a DRY piece of cotton or gauze, wipe away the first drop of blood.

8) Pressing the finger firmly, apply 3 drops of blood (size •) at one end of the slide for thick smear and about 1 cm away, apply 1 drop (this size •) on the slide for thin smear. ***When applying the blood on the slide, avoid the finger to touch the slide***. (See figure 7. 5).

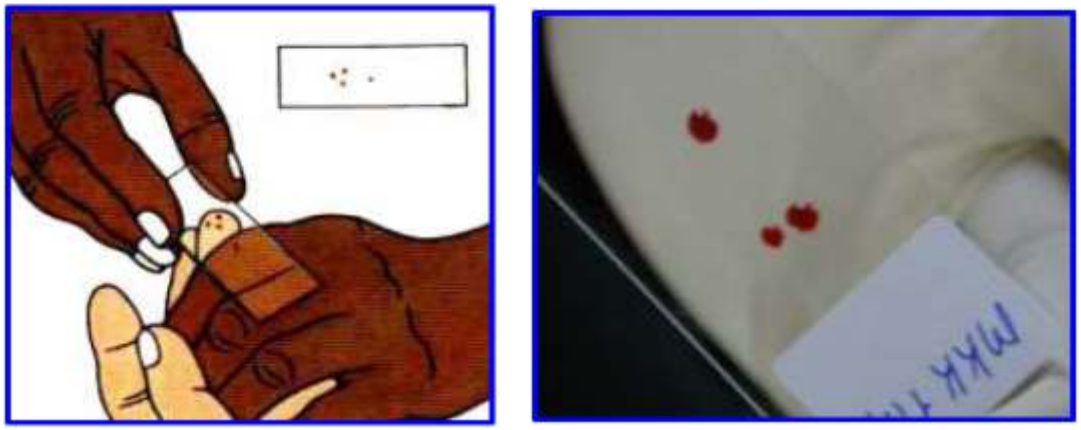


Fig 7. 5

! The quantity of blood is very important for the thick smear (See figure 7. 5)! Not enough blood may lead to a WRONG NEGATIVE RESULT.

9) Use a second slide (called spreader) to spread the big blood drop for thin smear. Do it with a slow and regular movement to make a good blood film.

- Put the spreader on the first slide and draw it backward
- Let the blood run across the edge of the spreader. The spreader must have a smooth & cleaned edge otherwise it is not possible to obtain a good thin smear.

- Firmly push the second slide, keeping it at an angle of 45 degrees, with a slow and regular movement until it is off the first slide (see figure 7. 6).

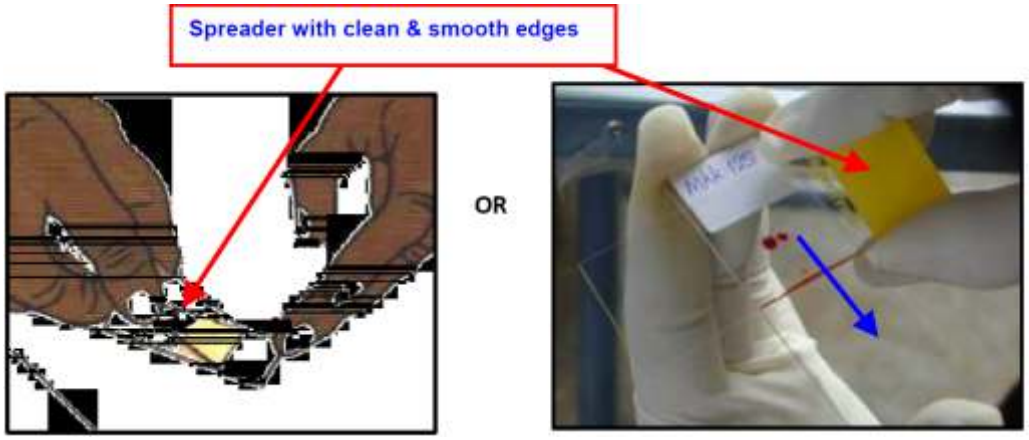


Fig 7. 6

10) If the smear is good prepare the thick film:

Quickly join the 3 small drops of blood and mix them circularly not less than 10 times by using the corner of the spreader (see figure 7.7). Then use an alcohol soaked cotton to wipe the blood off the spreader.

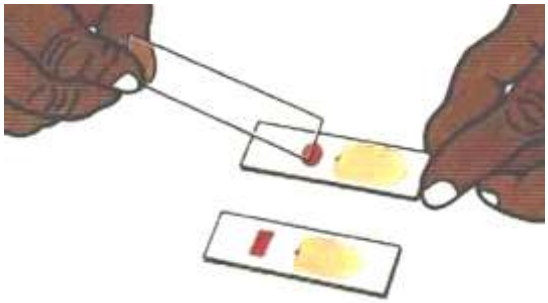


Fig 7. 7

11) Let the slide dry on a plane surface.

- Label the slide with the patient's number and date: use a PENCIL.

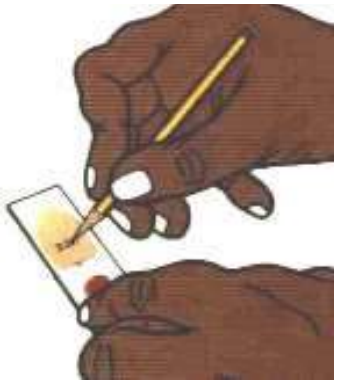


Fig 7. 8

Malaria rapid test

- Malaria rapid diagnostic tests (RDTs) assist in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood.
- RDTs are an alternative to diagnosis based on clinical grounds or microscopy, particularly
- Where good quality microscopy services cannot be readily provided. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites in the blood of infected individuals.
- Some RDTs can detect only one species (Plasmodium falciparum or P. vivax) while others detect multiple species (P. falciparum, P. vivax, P. malariae and P. ovale). Blood for the test is commonly obtained from a finger-prick.
- Testing kit may be available in cassette/ strip.
  Procedure: follow instructions given in the kit insert.

9.3 FIXING AND STAINING

A. FIXING

1) Materials needed:

- Absolute methanol
- Slide-rack

2) Method: After the blood smear completely dries:

- Fix only the thin smear by dipping it QUICKLY in methanol 3 – 5 seconds. Be careful because the vapour of methanol may fix the thick smear.
- Let the slide dry on the slide-rack completely before staining.

B. STAINING

1) Materials needed:

- Pure GIEMSA stain
- Clean filtered water with pH at or near 7.2
- Wash-bottle
- Graduated cylinder (100 ml)
- Graduated pipette (10ml)
- Slide-rack
- Staining tray

* *You may need to filter the pure Giemsa stain before using it (depend on the quality of stain). This should be prepared by shaking the original bottle of pure Giemsa stain first then filter the stain using a filter paper into another dry clean bottle and tighten the lid (Giemsa contains methanol)*

* *We cannot stain the smear with pure Giemsa BEFORE STAINING WE HAVE TO DILUTE GIEMSA WITH FILTERED WATER in order to obtain a 10 % Giemsa solution.*

* *The percentage of Giemsa in the solution may vary. It depends on the quality of the water, the pure Giemsa stain and the use of filtered or non-filtered pure Giemsa stain. You must be able to recognize good stain from bad stain in order to adjust the appropriate dilution.*

2) How to dilute Giemsa stain for 10 % dilution (for 20 – 30 slides):

- Fill about half of 100 ml cylinder with filtered water.
- Add exactly 10 ml of filtered Giemsa into the water by using dry pipette.
- Complete exactly the cylinder to the mark 100 ml with filtered water.
- Pour into separate clean and dry wash bottle.
- This 100 ml of the solution is enough for 20 – 30 slides.
- Clean your equipment after use.

* Staining one slide needs about 3 to 5 ml of Giemsa solution. The quantity of solution prepared depends on the amount of slides.

3) How to stain:

- Set the timer for 20 minutes and cover the first slide with the Giemsa solution
- Then cover the 2nd, 3rd, 4th…slides.
- Let the solution remain on the slides for 20 minutes.
- Wash slides with filtered water following the same order as for the staining; 1st, 2nd, 3rd, 4th … slides by pouring the water gently on the slides from the thin smear-end and then tip them up.
- Let the slides dry on the slide-rack.

9.4 PRINCIPLE of SMEARING, FIXING and STAINING

A. SMEARING

Thin smear : A thin smear is prepared by spreading a small drop of blood evenly on a slide so that it forms only one layer of cells (see figures 7. 9 & 7. 10).

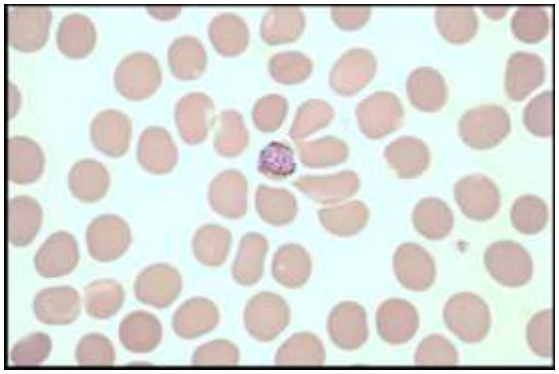


Fig 7. 9: P.malariae gametocyte on the thin smear

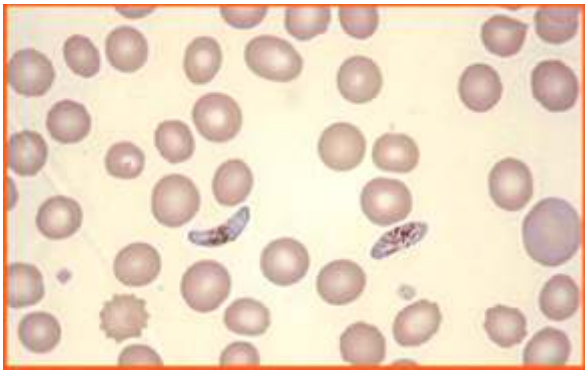


Fig 7. 10: P.falciparum gametocytes on the thin smear.

Thick smear : A big amount of blood on a small surface makes it possible to find parasites more quickly and easily when only a few parasites are present (see figure 7. 11).

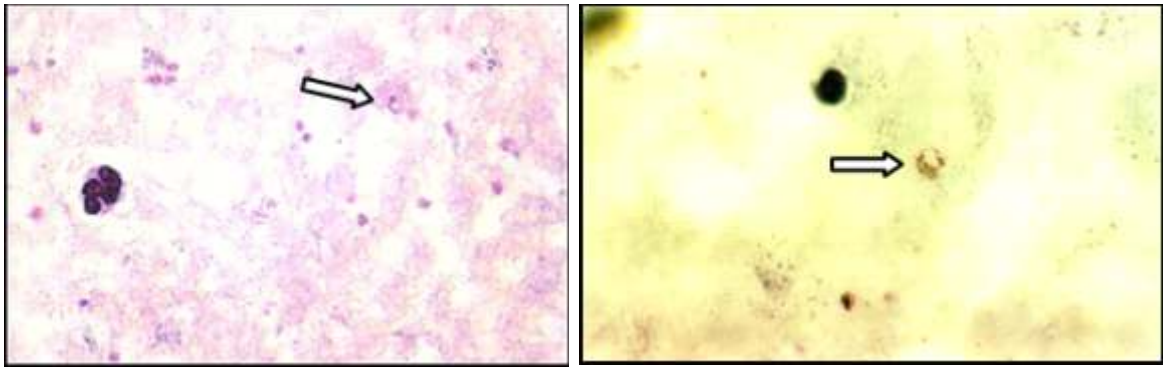


Fig 7. 11: Plasmodium on thick smear

B. FIXING

Thin smear : To ensure that RBCs are not destroyed by water (from the staining solution) and to attach the film securely to the slide. Methanol preserves and protects cells. ***!!! Water destroys the cells, so***

- Be careful not to have water mixed with methanol, close the bottle of methanol properly.
- During the rainy season, change the methanol more often.

Thick smear : Must not be fixed.
!!! Methanol fixes cell membrane, so

- Do not let methanol touch the thick smear.
- Fixing time must be as short as possible (about 3 – 5 seconds). If it takes too long vapours of methanol may fix the thick smear.

C. STAINING

Thin smear : To colour blood cells and parasites inside blood cells.

Thick smear : During staining, Red Blood Cell membranes are broken by water in the staining solution (see figure 7. 12).

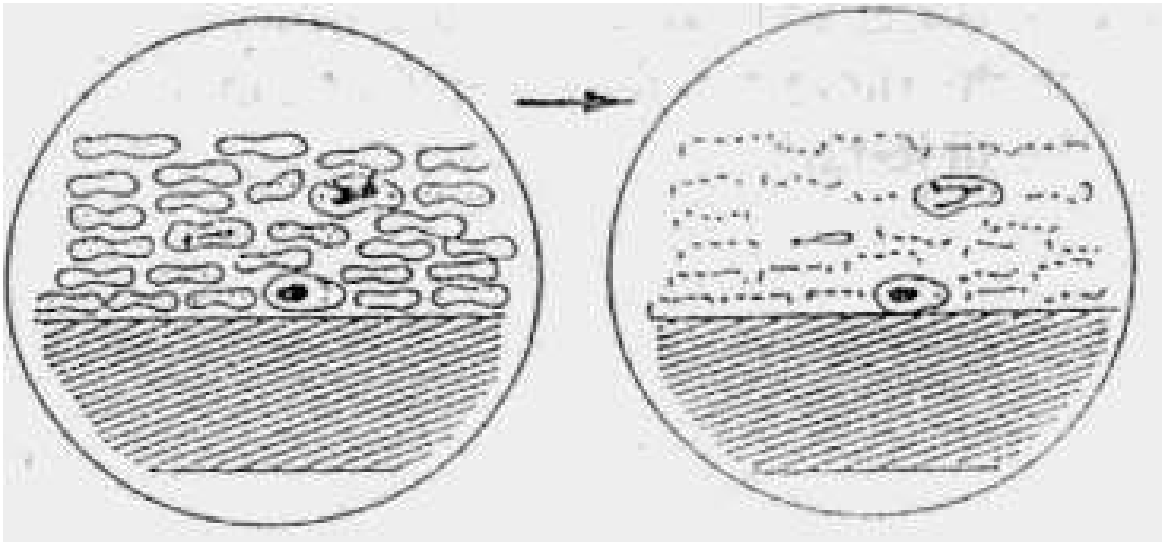


Fig 7. 12

9.5 HOW TO RECOGNIZE A GOOD STAIN

A good stain is necessary in order to have a good result. A bad stain will cause a bad result and an incorrect treatment. So you must be able to manage your stain quality.

A. CRITERIA FOR A GOOD STAIN

Colour of the blood cells and malaria parasites after Giemsa staining:

Thick smear:	WBC nucleus:	deep purple
	WBC cytoplasm:	should not be seen
	RBC cytoplasm:	hould not be seen (if you can see it, it means That the thick smear was fixed with methanol)
	Platelets:	pink
	Background:	pale grey (derived from lysed RBCs) and free from dust. **Blue background means that the stain is bad**.
	Nucleus of malaria parasite:	deep red
	Cytoplasm of malaria parasite:	deep blue
Thin smear:	WBC nucleus:	deep purple
	WBC cytoplasm:	blue or pale pink
	RBC cytoplasm:	grey – pink to pale purple
	Platelets:	pink
	Back ground:	free from dust and clear
	Nucleus of malaria parasite:	red
	Cytoplasm of malaria parasite:	blue

B. CAUTION for HAVING A GOOD STAIN

All the technical steps from smearing to staining are very important.

1) Blood smear:

- Use well cleaned slides.
- A standardized thick smear is needed: thick smears with too much blood cannot be stained properly.
- Thin smears should be spread evenly by using a good spreader (smooth edged slide) and a proper quantity of blood.
- You must allow the thick smear to dry thoroughly; otherwise it will not stain properly.

2) Fixing:

- Allow the blood smear to dry completely on a plane surface before fixing
- Fix only the thin smear by dipping it quickly in methanol.
- Let methanol dry completely before staining.

3) Preparing the Giemsa dilution:

- Filter the pure Giemsa before using it. Shake it well before filtering.
- Use clean and dry instruments for preparing dilution (cylinders, pipettes, etc.).
- Make an accurate dilution.
- Do not use this dilution if it is more than 2 hours since preparation.
- Rinse the instruments very well with filtered water after washing them:
- Never mix soap with reagents or filtered water.

4) Staining:

- Stain out of direct sunlight.
- Stain for exactly the amount of time needed.
- For washing the stained slides, pour clean water gently on the slide before draining the stain from it. This will protect the smear from Giemsa precipitation. Do not pour water directly on the thick smear side (the blood might be removed from the slide).
- The proportion of Giemsa dilution and staining time should be adjusted before using any new batch of stain or new manufacturer.

5) Quality of water:

- To have a good stain, you need neutral water with pH about 7.2 (neither too acid nor too alkaline).
- The water must be clean and must be filtered.

Note: pH is the symbol for the degree of acidity or alkalinity of the solution; pH values from 0 to 7 indicate acidity and from 7 to 14 indicate alkalinity. The pH can be found by using pH indicator paper (litmus paper)

C. WHAT TO DO IF YOU HAVE A BAD STAIN

► First, check that every technical step are done properly:

- Blood smear Fixing
- Giemsa dilution Staining
- The washing of staining instruments and storage

► If they are properly done and you still have a bad quality stain, it might be due to bad water quality (pH not adequate) or a bad batch of Giemsa. So try to change the water or use a new batch of Giemsa.

► Sometimes you only have few bad slides in a day. In this case, repeat the smear. If not possible, you might re – stain those slides by washing them with methanol. Remove the old stain then let those slides completely dry and carefully stain them again.

9.6 EXAMINATION of BLOOD FILMS for MALARIA PARASITES

A. Recognition of a malaria parasite

Malaria parasites take up Giemsa stain in a special way in both thick and thin blood films. You must be able to distinguish the various parts of the parasite especially from the thin blood film as shown in figure 7. 13.

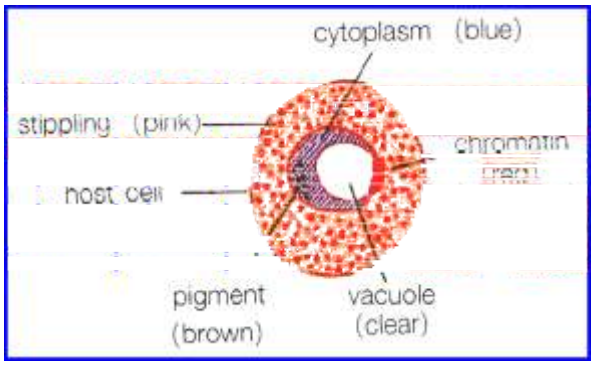


Fig 7. 13: Parts of a malaria parasite inside a red blood cell

Malaria parasites develop through many stages; however, in all stages the same parts of the parasite will stain the same color:

Chromatin (part of the parasite nucleus) is usually round in shape and stains deep red.

Cytoplasm is formed in various shapes from a ring shape to an irregular shape. It always stains blue.

B. Stages of the malaria parasite

I The Trophozoite stage

This stage is the most commonly seen; often called the ring stage form even sometimes it is an incomplete ring (see figure 7. 14).

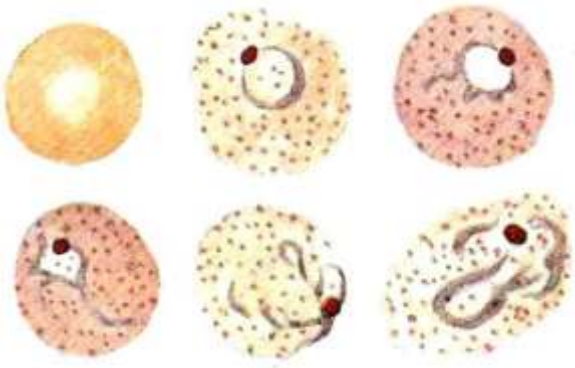


Fig 7. 14: The trophozoite stage of P.vivax

Because the trophozoite stage is a growing stage, the parasite within the red blood cell may vary in size. Pigment appears as the parasite grows. Malaria pigment is a by-product of the growth of the parasite. It does not stain, but has a color of its own, which may range from pale yellow to dark brown or black.

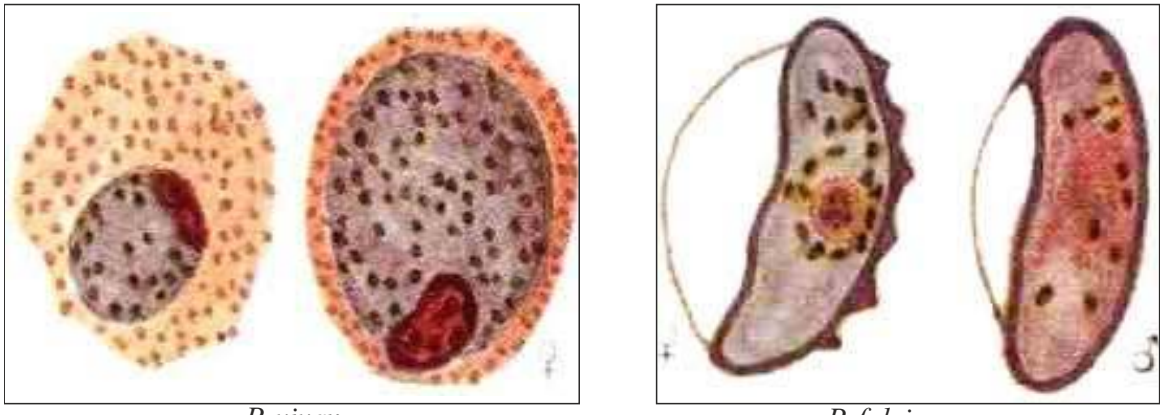
II The Schizont stage

At this stage, the parasite reproduces itself by simple division so its chromatin may be seen from two pieces to a certain number of chromatin dots as shown in figure 7. 15

Fig 7. 15: The schizont stage of P.vivax

III The Gametocyte Stage

Gametocytes may be either round or banana – shaped, depending on the species (see figure7. 16).



P. vivax *P. falciparum*

Fig 7. 16: Male and female gametocytes

C. Species of malaria parasite

Appearance of parasite species in thin blood films

After knowing how to recognize malaria parasite and their stages in thin blood films, you need to distinguish the species of malaria. The simplest guide is the effect the parasite has on infected red blood cells. These are:

SIZE of the RBC:

* No change for PF infections.
* No change or smaller for PM infections.
* Enlarged for PV infections.
* 20 to 30 % of parasitised RBC becomes slightly enlarged and oval in shape, with fimbriated (ragged) ends for PO infections.

COLOR of the RBC:

* No change for PF infections.
* No change or darker for PM infections.
* More pale for PV and PO infections.

DOTS (in RBC cytoplasm): * No dots for PM infections.

* Sometimes few coarse and irregular purple – red dots called "Maurer's dots" in PF infections.
* Usually numerous small pink round dots scattered all over the RBC cytoplasm for PV infections. These dots are called "Schuffner's dots".
* Usually numerous dark dots called "James's dots" in PO infections. They appear brighter and larger than "Schuffner's dots".

You should consult the 4 color plates of "Appearance of Plasmodium parasite stages in Giemsa – stained thin and thick blood films" produced by WHO (see Figure 7.17a to figure 7.17d). The left hand side of each plate shows the various stages of each malaria parasite species as they appear in thin blood films, and the right hand side shows their appearance in thick blood films.

Appearance of parasite species in thick blood films

Not only the appearance of both red and white blood cells differs in thin and thick blood films; there are also differences in the appearance of malaria parasites. These are:

- No red blood cells seen in thick blood film but the ghosts of red cells can sometimes be seen surrounding parasites in the thinner parts of the films.
- Like the WBCs, the malaria parasites appear to be smaller in thick blood films than in thin blood films.
- The fine rings of cytoplasm of the trophozoites may appear incomplete or broken.
- The schuffner's dots sometimes are seen in thick blood films.
- The Maurer's dots of PF sometimes are seen in thick blood films.

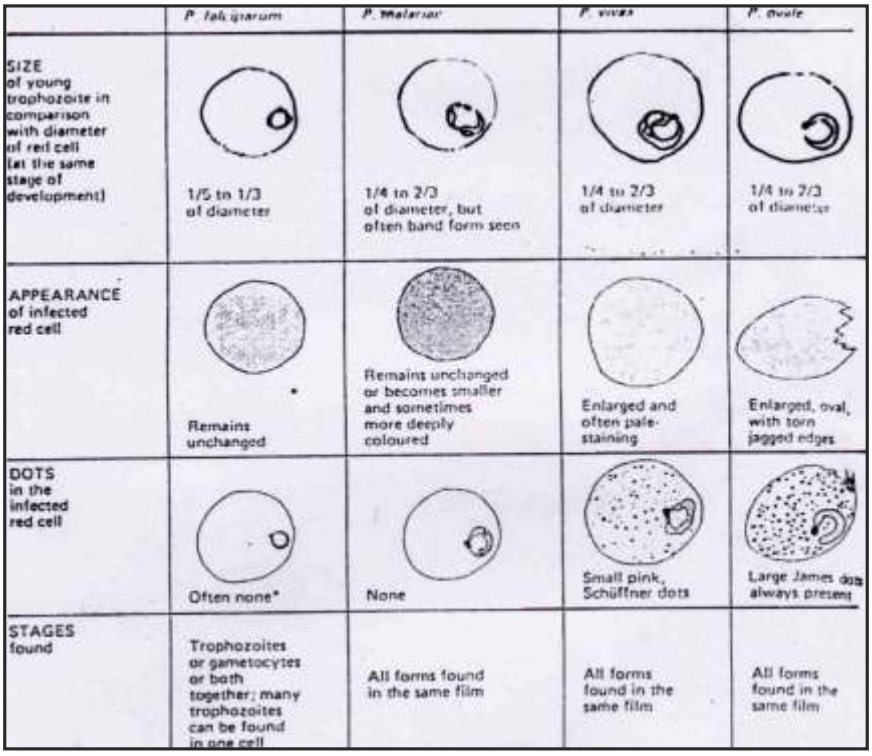
By looking at the thick films, you can find out if the slide is negative or positive.

You can also estimate the amount or density of malaria parasites by reporting in the number of plus signs: 1+, 2+, 3+ or 4+.

COMPARISON OF INFECTED RED BLOOD CELLS IN THE THIN BLOOD FILMS

	P. falciparum	*P. malariae*	*P. vivax*	*P. ovale*
Size of **young** trophozoite in comparison with diameter of red blood cell (at the same stage of development)	*1/5 to 1/3 of RBC diameter*	*1/4 to 2/3 of RBC diameter*	*1 / 4 to 2/3 of RBC diameter*	*1 /4 to 2/3 of RBC diameter*
APPEARANCE: of infected red cell	*Remains unchanged*	*Remains unchanged or becomes smaller and sometimes more deeply colored*	*Enlarged and often pale staining*	*Slightly enlarged, oval with jagged edges*
DOTS: in the infected red cell	***Big and few*** *blue-mauve Maurer's dots in older trophozoites*	***None***	***Small and numerous*** *pink, Schuffner's dots*	***Big and numerous*** *deep brown James' dots*
STAGES found:	*Trophozoites and/or gametocytes. Schizonts rarely seen in simple malaria.*	*All forms might be found in the same film*	*All forms might be found in the same film*	*All forms might be found in the same film*
PARASITAEMIA	*Up to 50 % of RBCs infected*	*Rarely more than 1 % of RBCs infected*	*Rarely more than 2 % of RBCs infected*	*Rarely more than 2 % of RBCs infected.*

COMPARISON OF INFECTED RED BLOOD CELLS IN THE THIN BLOOD FILMS



***Note*: Keep in mind there is Maurer's dots in older trophozoites of P.falciparum.**

COMPARISON OF EACH TYPE OF MALARIA PARASITES IN THIN BLOOD FILMS

		P. falciparum	*P. vivax*	*P. malariae*	*P. ovale*
Trophozoites	Young				
	Old				
Schizonts	Immature				
	Mature				
Gametocytes	Male				
	Female				

COMPARISON OF EACH TYPE OF MALARIA PARASITES IN BOTH THICK AND THIN BLOOD FILMS.

Fig 7. 17a: ***Plasmodium falciparum***

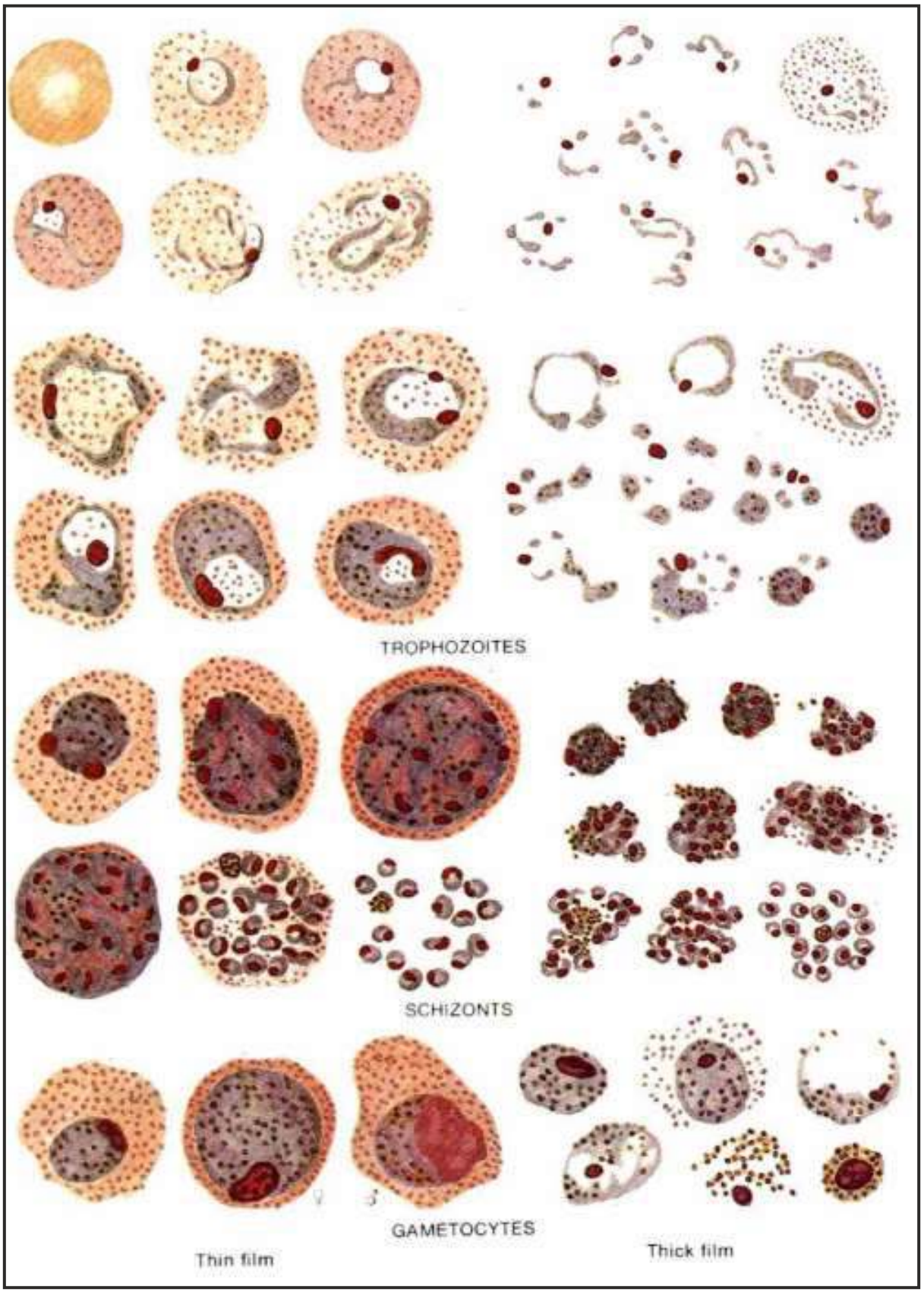


Fig 7. 17b: ***Plasmodium vivax***

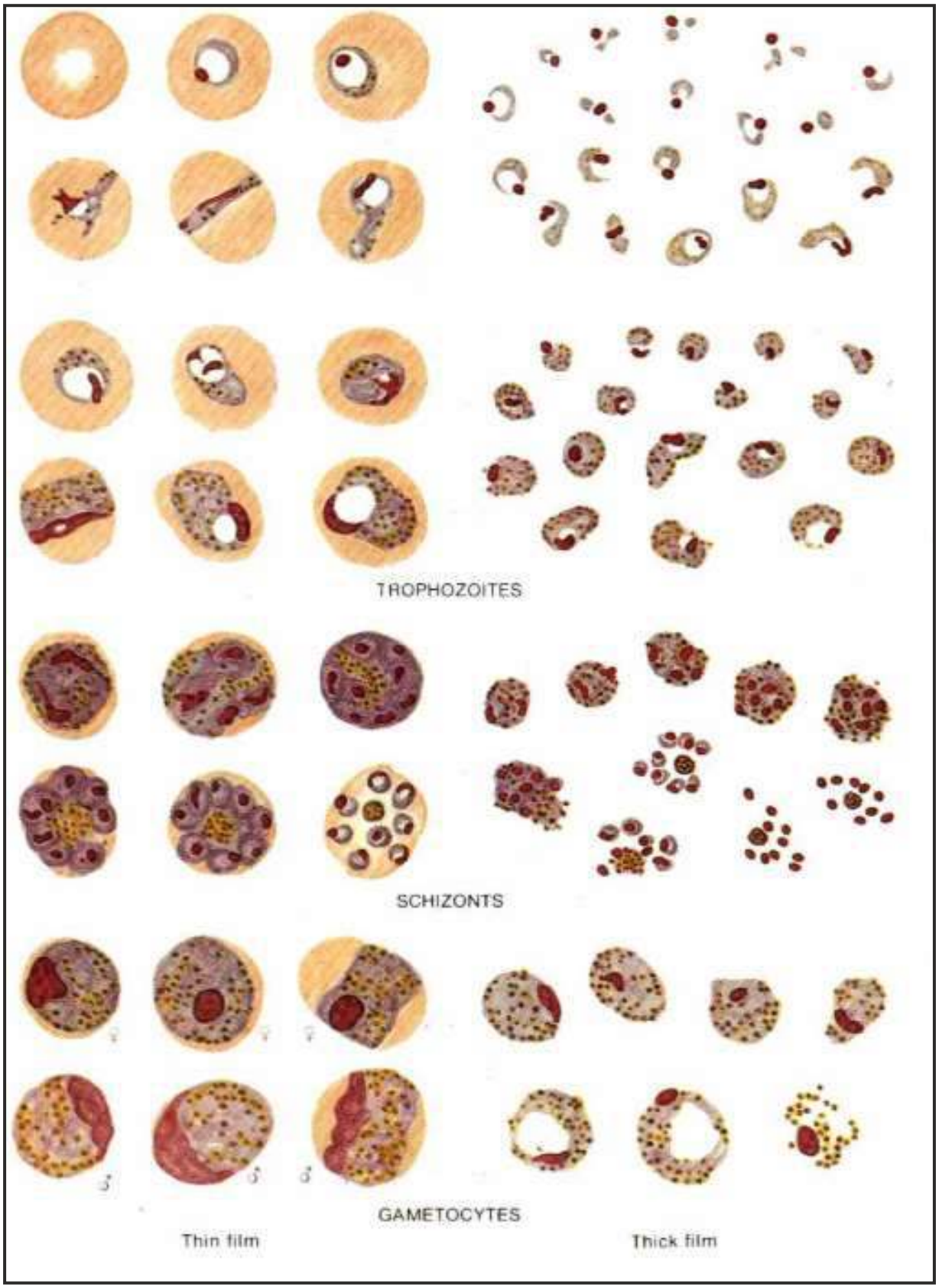


Fig 7. 17c: ***Plasmodium malariae***

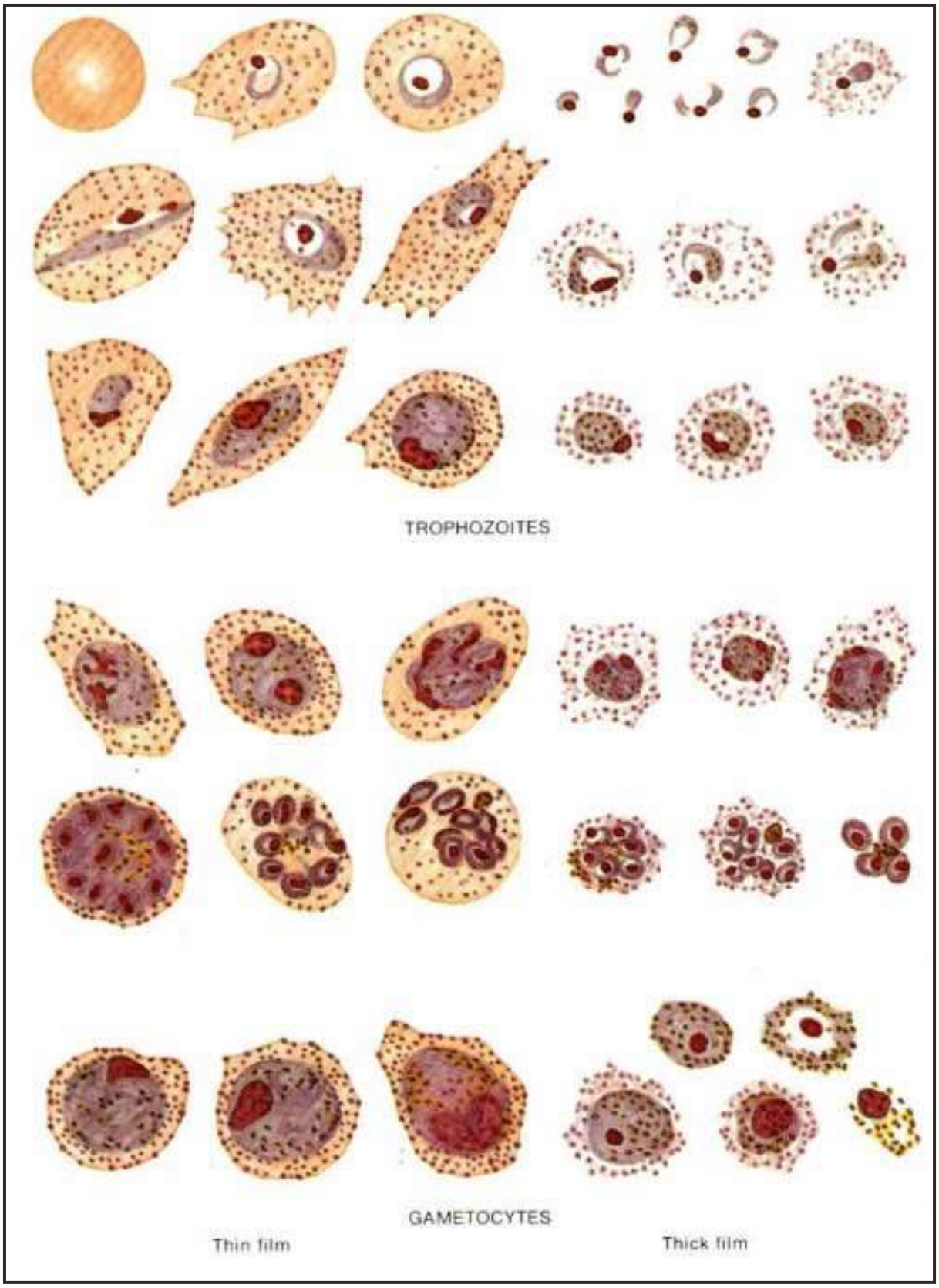


Fig 7. 17d: ***Plasmodium ovale***

9.7 ROUTINE EXAMINATION OF BLOOD FILMS FOR MALARIA PARASITES

A. Examining the thin film :

Since it takes 20 to 30 times as long to examine a thin film as to examine a thick film, routine examination of thin films is not recommended except in these following circumstances:

- When it is necessary to confirm the identification of a specie.
- When estimation of the percentage of RBC infected is needed (see lesson 7.8).
- When the thick smear is invalid and you cannot repeat the smear. The thick smear might not be possible to examine because it is too small, or it has become fixed, or it is wiped out but it is preferable to repeat the smear.

NEVER GIVE A NEGATIVE RESULT BY LOOKING AT THIN SMEAR ONLY!

B. Examining the thick film :

Routine examination for malaria parasites is done on the thick blood films. However, sometimes it is difficult to differentiate some stages or species of malaria parasites so it needs to be confirmed by examination of the thin blood film.

The examination of each blood film should be done in a systematic way as follows:

1. Use X100 objective (with oil immersion).
2. Start looking from the edge of the middle of the film (as shown in figure 7.18).

Fig 7.18: Examination of a thick and thin blood films

3. Examine the blood film, following the pattern of movement shown one by one field systematically.
4. Continue the examination for 100 good fields to determine whether the blood film is positive or negative for malaria.
5. If doubtful diagnosis, more fields should be examined, or a second smear requested.
6. At the end of the examination, record the result on the lab request form and in the record book. The result must include parasite specie(s), stage(s) and density.

9.8 MALARIA PARASITE DENSITY

It is necessary to know the density of parasites in blood film because of these following reasons:

- To know how severe the malaria is.
- To know if the patient will need a blood transfusion.
- To know whether the parasites are responding to the antimalarial treatment being given.
- To know the severity of malaria infections being seen in the camp.

REPORT:

Parasites density is estimated on the thick smear by counting parasites in each field and then averaging the number and reporting as:

< 1 parasite / field	rare
1 – 2 parasites / field	+ or 1+
3 – 25parasites / field	++ or 2+

In order to get an accurate result; the number of parasites should be counted only from the standardized thick smear (see figure 7. 19) in which WBCs are regularly scattered.

ON A GOOD SMEAR, YOU SHOULD FIND ABOUT 10 WBCS PER FIELD

Bad smear
(not enough blood)

Fig 7. 19:

Good smear
(enough blood)

Sometimes the smear looks good with proper amount of blood but it is not spread regularly. Therefore some parts are thinner than others. In this case, you should count only in the good parts (see about 10 WBCs / field).

If the smear is too thick (too much blood), Giemsa stain is not able to enter the blood cells. The colour will be bad (blue) and you cannot see the parasites. In this case a new smear should be prepared.

PERCENTAGE OF INFECTED RBCs

This percentage is estimated on the thin smear

By definition a PFT 4+ is when 4 % or over 4% of the RBCs are infected.

IN ALL PATIENTS WITH PFT 3+ OR PFT 4+, THE PERCENTAGE OF RBCs PARASITISED MUST BE ASSESSED on the thin smear because:

* Treatment for patients with PFT 3+ or PFT 4+ might be different.

* Sometimes it is not easy to determine on the thick smear whether it is a PFT3+ or a PFT 4+, the percentage of RBCs parasitised will give you the accurate result (see definition of PFT 4+ above).

* In a PFT 4+ case, it is important for the medic to know if the patient has 5 %, 10 %, 20 % or 40 % of RBC parasitised. The management of the patient will be partially based on this result.

HOW TO DO:

1. Have a GOOD thin smear where RBCs are regularly spread. If not the case repeat a couple of thin smear.
2. Check first the thin smear with objective x 40 (without immersion oil) and find a place where RBCs are regularly spread: RBCs not overlapping or not too far away from each other. This is important for the accuracy of the result.

   IN ONE HIGH POWER FIELD (HPF: with objective x 100) WHERE RBCs ARE REGULARLY SPREAD, THERE IS APPROXIMATELY 200 RBCs.

   Important remark: This is valid for the Olympus microscopes (models CHD, CH-2, CHS and their respective components) widely used on this border.

   If others brands or models of microscope are to be used the average number of RBCs per HPF has to be assessed in the first place.
3. When the place is found, shift on the x 100 objective with immersion oil.
4. Count RBC with parasites (*) in 10 fields MINIMUM (**).

   * Note: One RBC infected with 3 parasites will be counted as 1 (not 3)

   ** Note: If the percentage is high (many parasites) counting 10 fields is enough to provide a good estimation. If the percentage is low (between 2 and 10%) there is fewer RBC with parasites to count. It is therefore recommended to count in 20 fields for a more accurate result.
5. Result

   If N is the number of RBCs with parasites counted in 10 fields.
   Then N is also the number of RBCs with parasites for 2000RBCs (1 field = 200RBCs)

   But we need a percentage, which is the number of RBCs with parasites for 100 RBCs.

   N Divided by 2 will be the number of RBCs with parasites for 1000 RBC, and then you need to divide again by 10 to find the number of RBCs with parasites for 100 RBCs.

   Note: you do not need a calculator to divide a number by 2 and then by 10.

   Example:

   You counted 252 RBCs with parasites in 10 fields (for 2000 RBCs)
   So you have 126 RBCs with parasites for 1000 RBCs.

   The result will be PFT 4+ with 12.6 % of RBC parasitised

Laboratory indicators of severity:

- Parasites 4+ or ³ 4% Schizonts
- Presence
- Malaria pigment in WBCs (monocytes and / orneutrophils):see figure7. 20.

!!! These should be reported to the medic !!!

Example of good results:

PFTS 4+; 12% of RBCs parasitised; presence of malaria pigment in WBCs.

PFTG 4+; 16% of RBCs parasitised.

Examples of bad results:

PFTS 4+

PFT4+; 3% of RBCs parasitised.

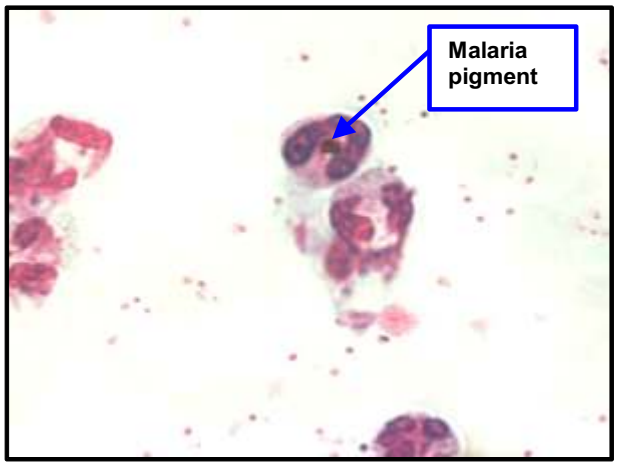


Fig 7.20: Malaria pigment inside a neutrophil (blue arrow).

9.9 MALARIA RECORD

FOR POSITIVE RESULT:

You must write:
- The name of malaria parasite types (PF, PV, PM, or PO)
- The stage of the development
  - T = Trophozoite
  - S = Schizonts
  - G = Gametocyte
- The parasite density (+, ++, +++, ++++ and/or percentage infected RBCs)
- Malaria pigment in WBCs

Example of malaria report
- PFT 1+
- PVTS 2+
- PFT 1+; PVT2 +
- PVT 2+; PMTS 1+

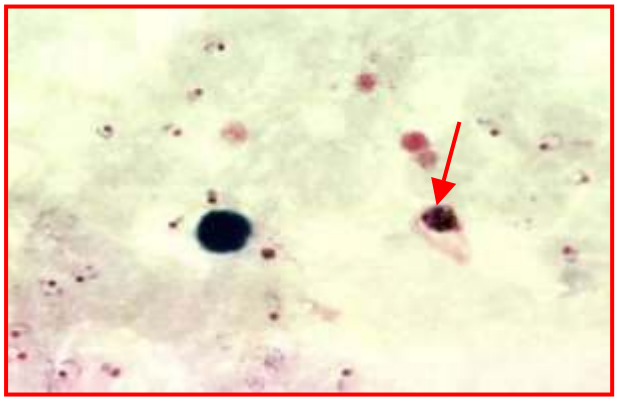
FOR NEGATIVE RESULT:

You must write: - NF (Not found) or Neg (Negative)

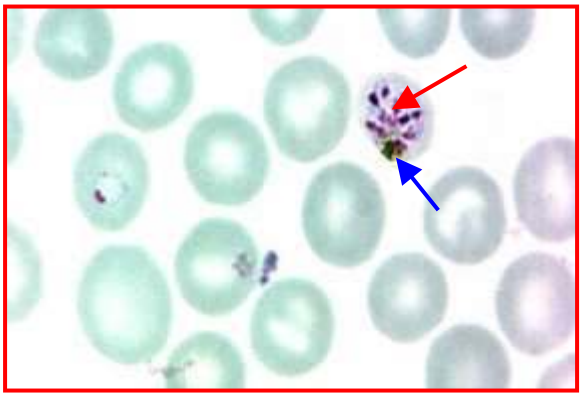
All of the following data must be written on the request form as well as in the malaria register book:

- Date
- Patient's name
- Patient's sex
- Slide number
- Patient's age
- Address of the patient
- Age group (A, B or A, B, C or whatever on your medical data system)
- Request test (MS or M/S)
- Examination result
- Signature.

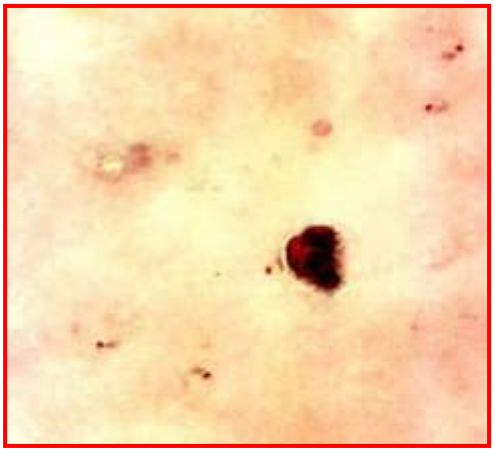
Pictures of *Plasmodium falciparum*



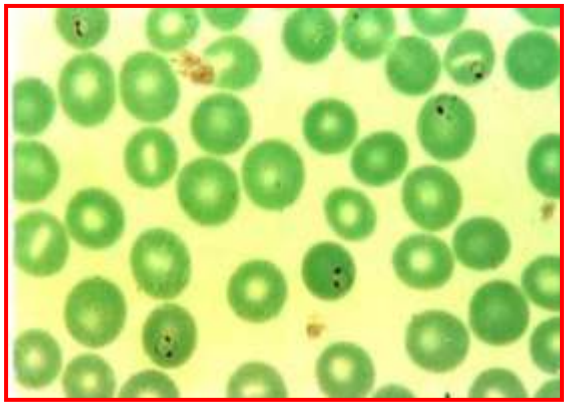
P.falciparum rings and schizont (arrow) on thick blood film.



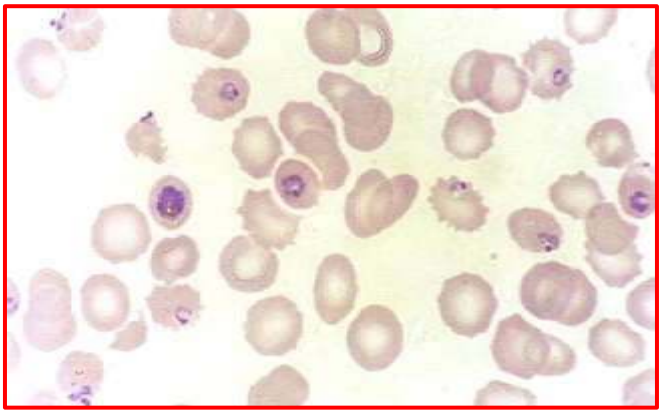
P.falciparum schizont (right) with merozoites (red arrow) and malaria pigment (blue arrow). One ring form on the left.



P.falciparum trophozoites on thick film.



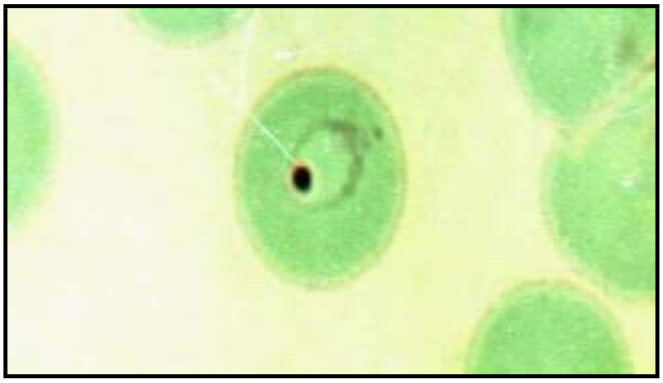
P.falciparum tiny rings on the thin film. Note the size of the ring compare to the RBC size.

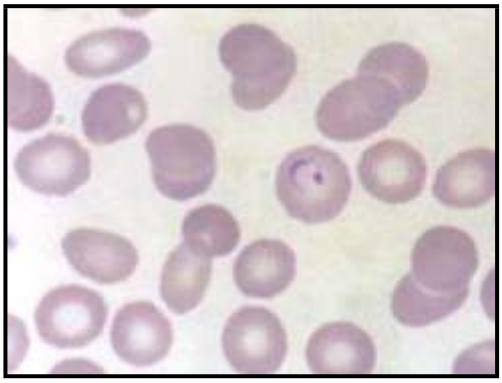


P.falciparum old trophozoites on thin film. Note the parasite cytoplasm is thicker and the RBC size remains unchanged.

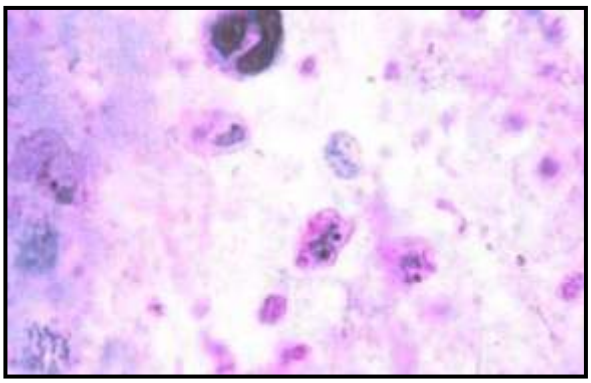
P.falciparum old trophozoites with 5 Maurer's dots in RBC cytoplasm (blue arrows).

Pictures of *Plasmodium vivax*

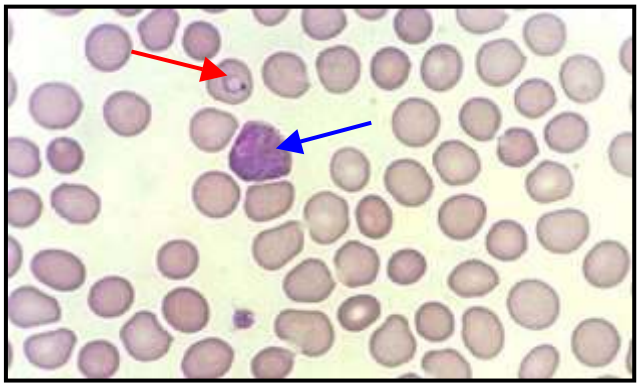




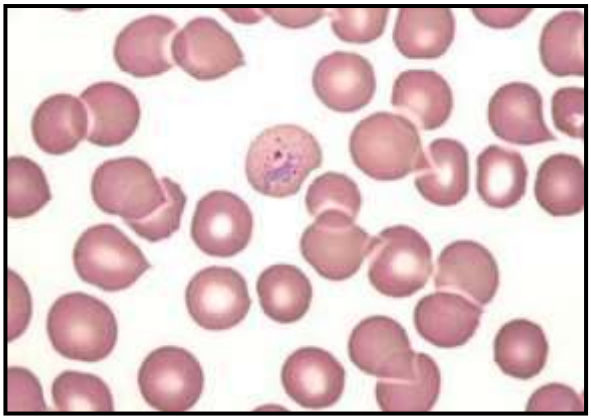
P. vivax young trophozoites on the thin film: infected RBCs are just very slightly enlarged but mostly; what makes the diagnosis is the size of the ring.



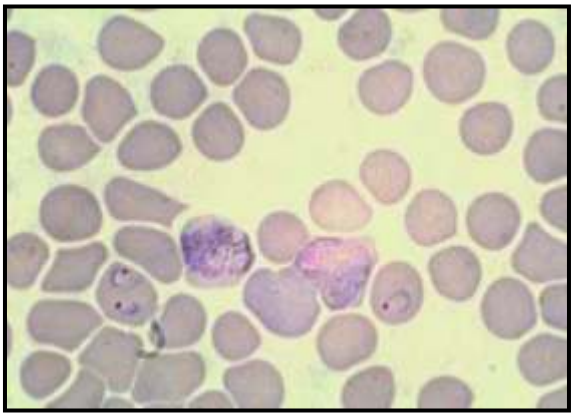
P. vivax old trophozoites on thick film: big rings with irregular cytoplasm.



P. vivax young ring (red arrow) and *P. vivax* gametocyte (blue arrow).

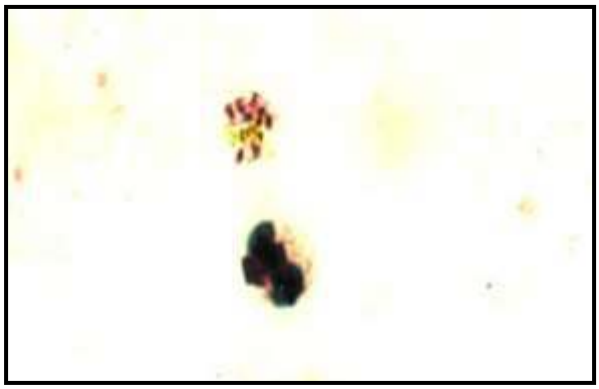


P. vivax old trophozoite with Schuffner's dots in RBC cytoplasm.

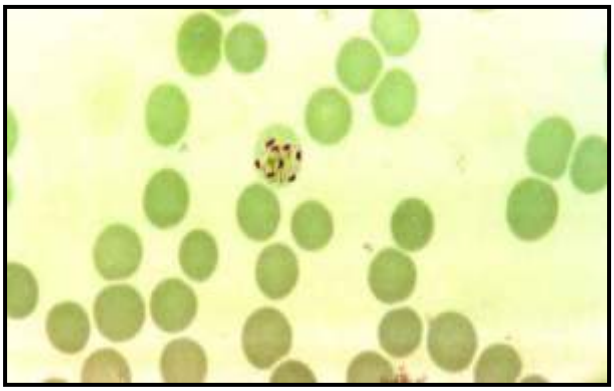


*P. v*ivax young rings (RBC on the left is infected with 2 rings) and gametocytes.

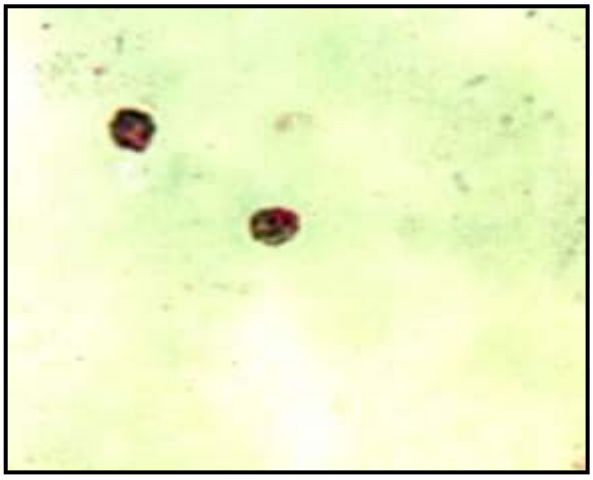
Pictures of *Plasmodium malariae*



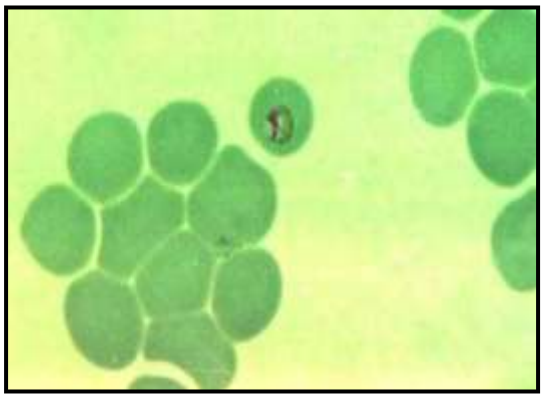
P. malariae schizonts on the thick film. Note the characteristic yellow-brown pigment.



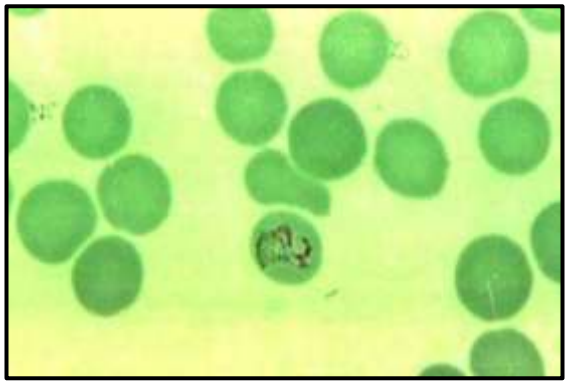
P. malariae schizont with 8 neatly arranged merozoites (rosette formation). RBC not enlarged and yellow-brown pigment



2 trophozoites of *P. malariae* on thick film. Characteristic dense and compact cytoplasm close to the nucleus

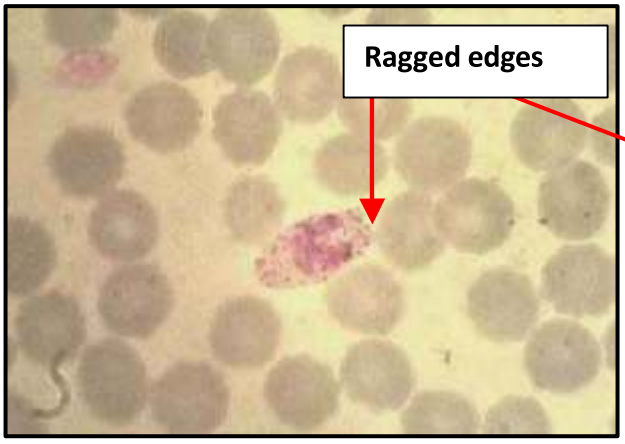


P. malariae on thin film. Note the RBC size is clearly smaller here.

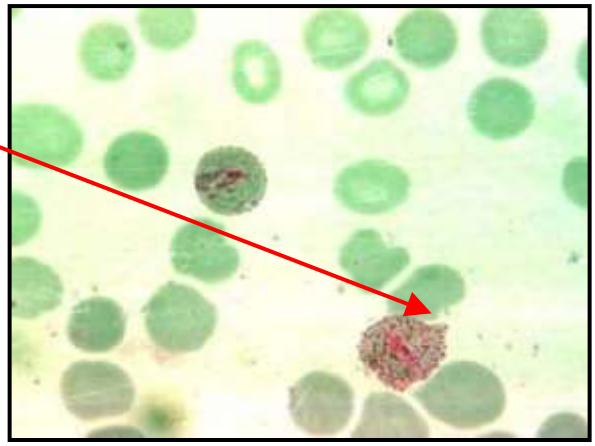


P. malariae trophozoite: typical **"band form"** across the RBC.

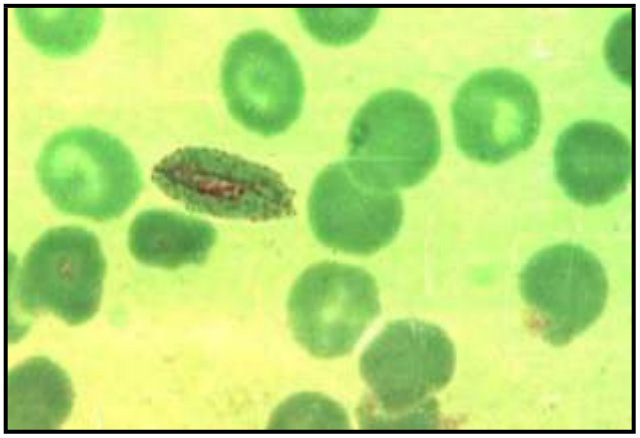
Pictures of *Plasmodium ovale*



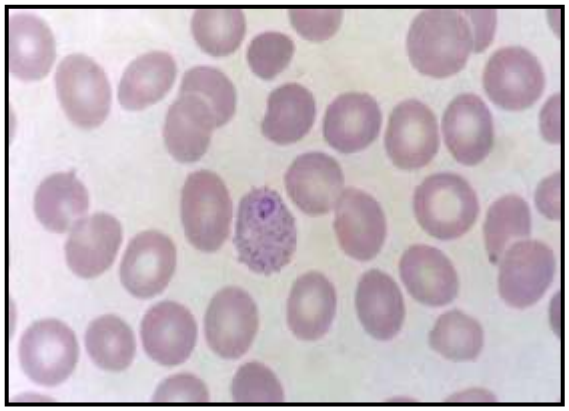
P. ovale old trophozoite on thin film. Note the oval shaped RBC with ragged (fimbriated) edges. RBC slightly enlarged compared to *P. vivax*. James' s dots are bigger and darker than Schuffner's dots.



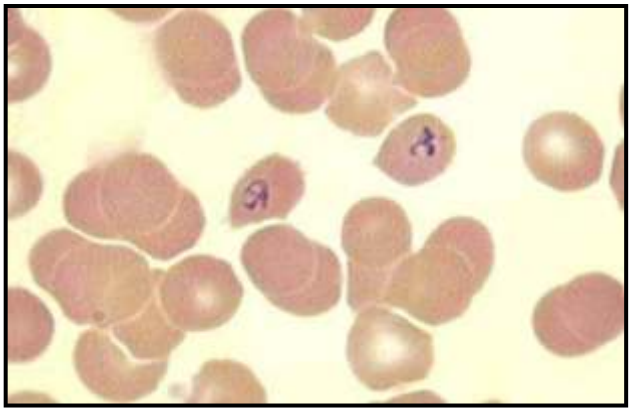
P. ovale old trophozoite (up) and gametocyte (down). The gametocyte is smaller than a *P. vivax* gametocyte and has ragged edges. The upper infected RBC is slightly enlarged but has a normal shape.



P. ovale old trophozoites on thin film. Both infected RBCs are oval shaped with ragged edges.

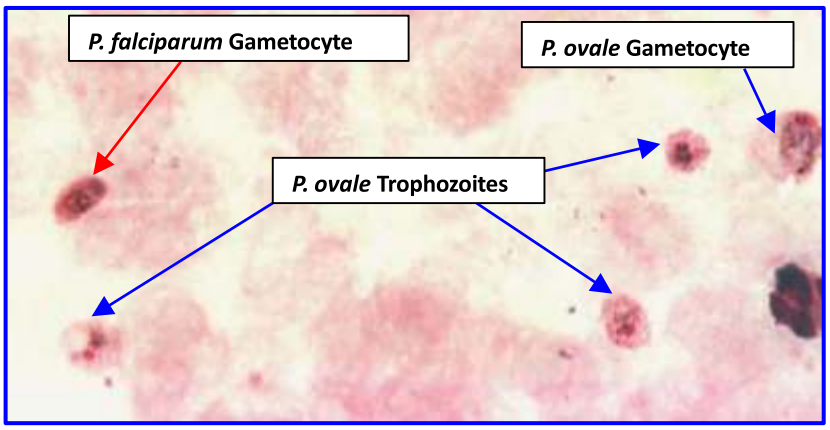


P. ovale young trophozoite (small ring). RBC is slightly enlarged and oval shaped. Note the very nice James's dots.

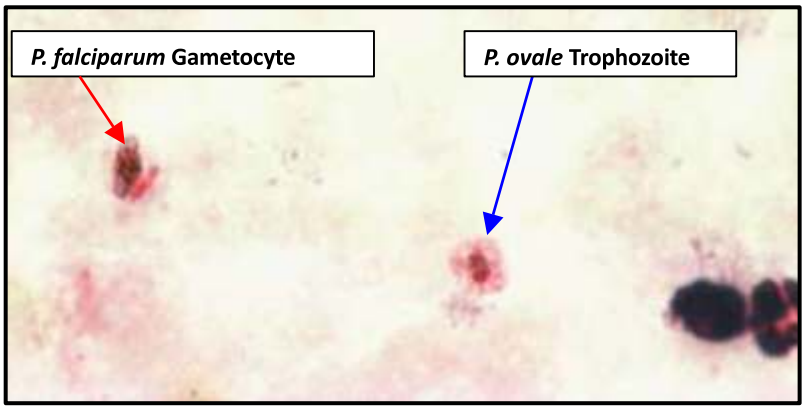


P. ovale young trophozoites. James's dots are invisible in the left RBC and barely visible in the right RBC because of the stain quality.

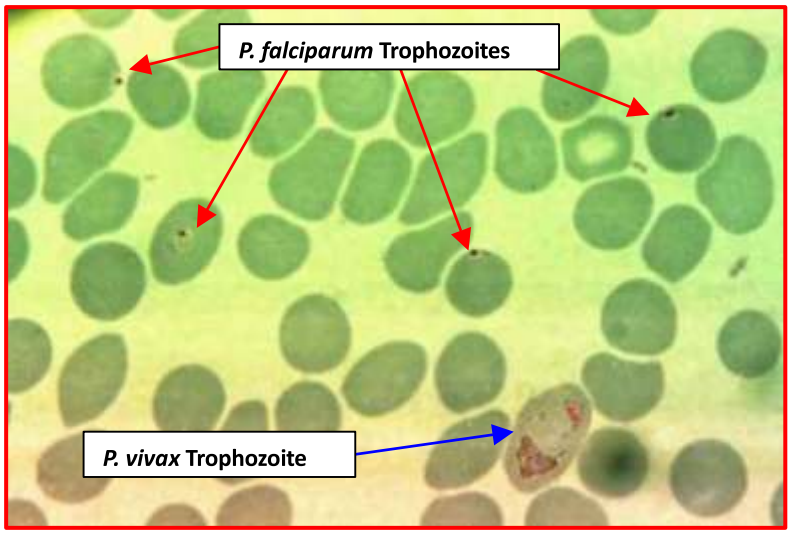
Pictures of mixed infections



P. ovale trophozoites on thick film are smaller than *P. vivax*. They also appear; like *P. malariae*, with dense and compact cytoplasm close to the nucleus: *P. ovale* needs to be confirmed on the thin film.



Same patient as above: *P. falciparum* trophozoites were not found. The result was POTG ++ and PFG Rare. Note that the *P. falciparum* gametocyte is more easily identifiable here.



P. falciparum trophozoites are young: small rings.
P. vivax trophozoites is old: infected RBC enlarged, parasite with big nucleus and irregular cytoplasm. Note that Schuffner's dots are present at that stage but are not visible on the picture.

CHAPTER - 10

FILARIASIS

Filariasis is a disease caused by parasites called **filarial worms**. Adult forms of these filarial worms live in the lymphatic tissues. Their young larvae live in blood circulation so we examine them in the same way as we do a malaria examination.

TRANSMISSION:

Transmission of these filarial worms is similar to malaria transmission by 3 types of mosquitoes including Anopheles.

SYMPTOMS AND CLINICAL SIGNS:

Only some infected persons develop clinical symptoms such as recurrent fever painful inflamed lymphatics.

The infection can cause thickened and rough skin on genital organs, breasts And limbs. The well-know sign is the appearance of the big lower leg (or foot) called "Elephant-foot" or "Elephantiasis"

DIAGNOSIS:

Filarial infections can be diagnosed by examining the blood. The young larvae called "microfilariae " are present in the blood and occasionally in urine. A high percentage of eosinophils can also be found.

The optimal time for collecting the blood sample is between 10:00 PM - 4:00 AM when the greatest number of microfilariae is present in the blood. Sometimes you may also find microfilariae during a regular malaria examination.

Thick blood smears stained with Giemsa are recommended for this exam, preferably using a bigger drop of blood than regularly used in the examination for malaria.

With a microscope, look for microfilariae which are:

- Very big compared with a malaria parasite: 200 to 300 um long large according to the species.
- They do not live inside the blood cells.
- They are big enough to be detected with X10 objective (see figure 8. 1), and then confirmed with X40 or X100 objective (see figures 8. 3 & 8. 4).

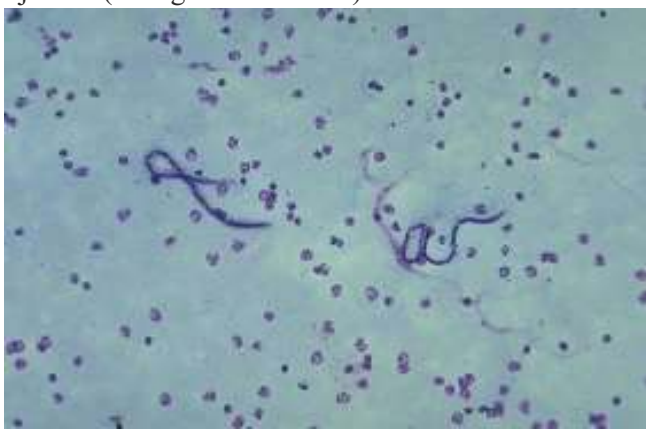


Fig 8. 1: Microfilariae as seen on a Giemsa stained blood smear with X10 objective.

Special characteristics of microfilariae are:

1 A colorless or pale sheath is often seen at both ends.

2 Nuclei are seen along the whole parasite body.

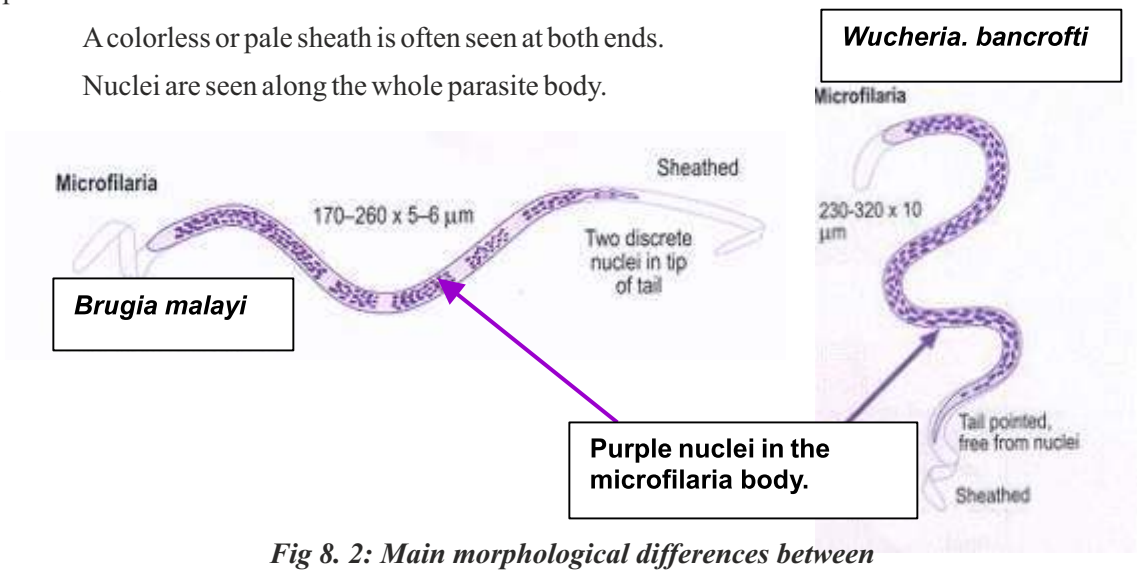


Fig 8. 2: Main morphological differences between the two species encountered in S.E Asia.

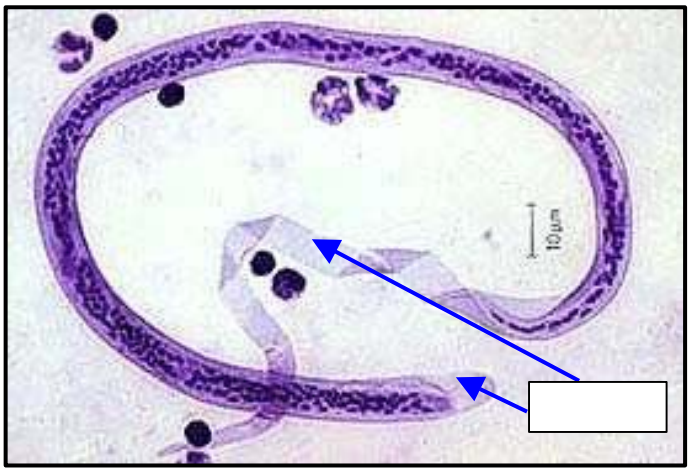


Fig 8. 3: *Wucheria bancrofti* microfilaria as seen with X40 objective. Note the pale sheath and the absence of terminal nuclei.

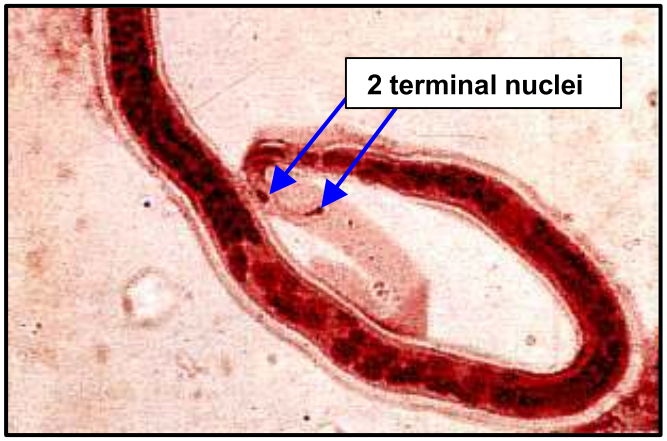
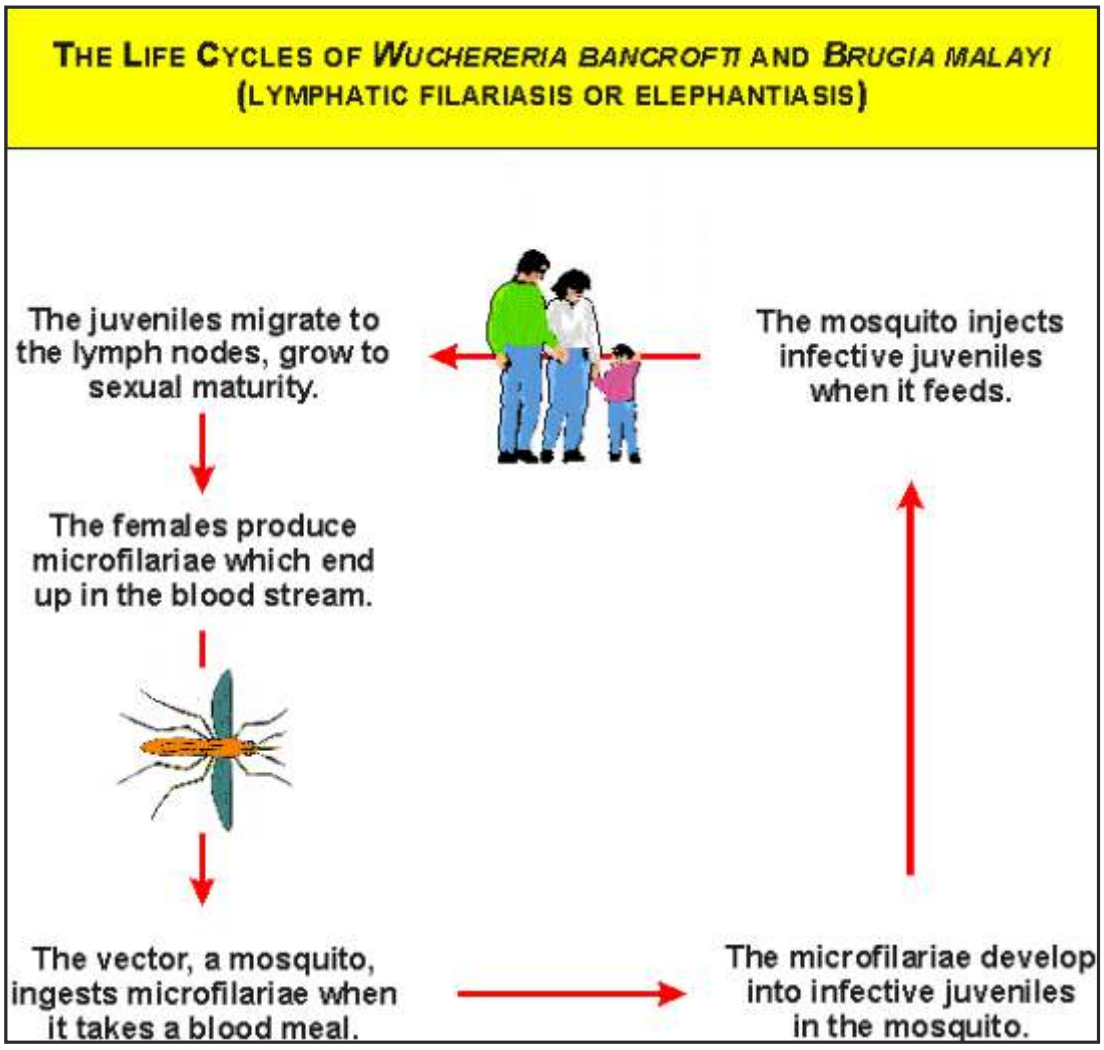


Fig 8. 4: *Brugia malayi* microfilaria as seen with X100 objective. Note the two terminal nuclei in the tail.

LIFE CYCLE OF FILARIA PARASITE



TREATMENT : with diethylcarbamazine (DEC)

People in endemic areas should check their blood for microfilariae once or twice yearly.

Filariasis- rapid test (strip method)

- Lymphatic filariasis is a parasitic disease, caused by microscopic, thread-like worms. The adult worms live in the human lymph system and release microscopic worms, called
- Microfilariae, into the blood. Approximately 90% of lymphatic filariasis is caused by W. bancrofti.
- The Filariasis Test Strip is an in vitro, immunochromatographic membrane assay for the detection of W. bancrofti antigen in capillary whole blood, collected via fingerstick.
- The test uses two different antibodies. One antibody is attached to colloidal gold and impregnated into a pink pad on the Test Strip. A second antibody is immobilized on the membrane.
- The blood sample is added to the lower half of the exposed white Sample Pad.
- The sample flows through the pink area of the pad and up the membrane, allowing any antigen in the sample to be labeled with the colloidal gold-labeled antibody. The antigen-antibody complex is then immobilized in the test result area of the Test Strip.

Procedure: follow instructions given in the kit insert

CHAPTER - 11

TUBERCULOSIS

Tuberculosis (TB) is a disease caused by infection with BACTERIA, ***Mycobacterium tuberculosis*** or TB bacilli (bacilli = rod shape bacteria). Tuberculosis usually attacks the LUNGS, causing pulmonary TB (see figure 10. 1). The bacilli cause holes to form in the lung. TB can also affect the spine, hips, lymph nodes, kidneys and many other parts of the body.

Transmission:

- Tuberculosis is spread by people, NOT by insects or blood transfusion.
- Like the common cold, TB is spread through the air.
- Only people who are sick with pulmonary TB can spread the disease. When they cough, sneeze, talk, sing or spit; the TB bacilli inside their lungs come out to the air.
- A person can get these bacilli by breathing them into the lungs.
- most infected people never develop the disease because their bodies have systems to fight against TB bacteria.

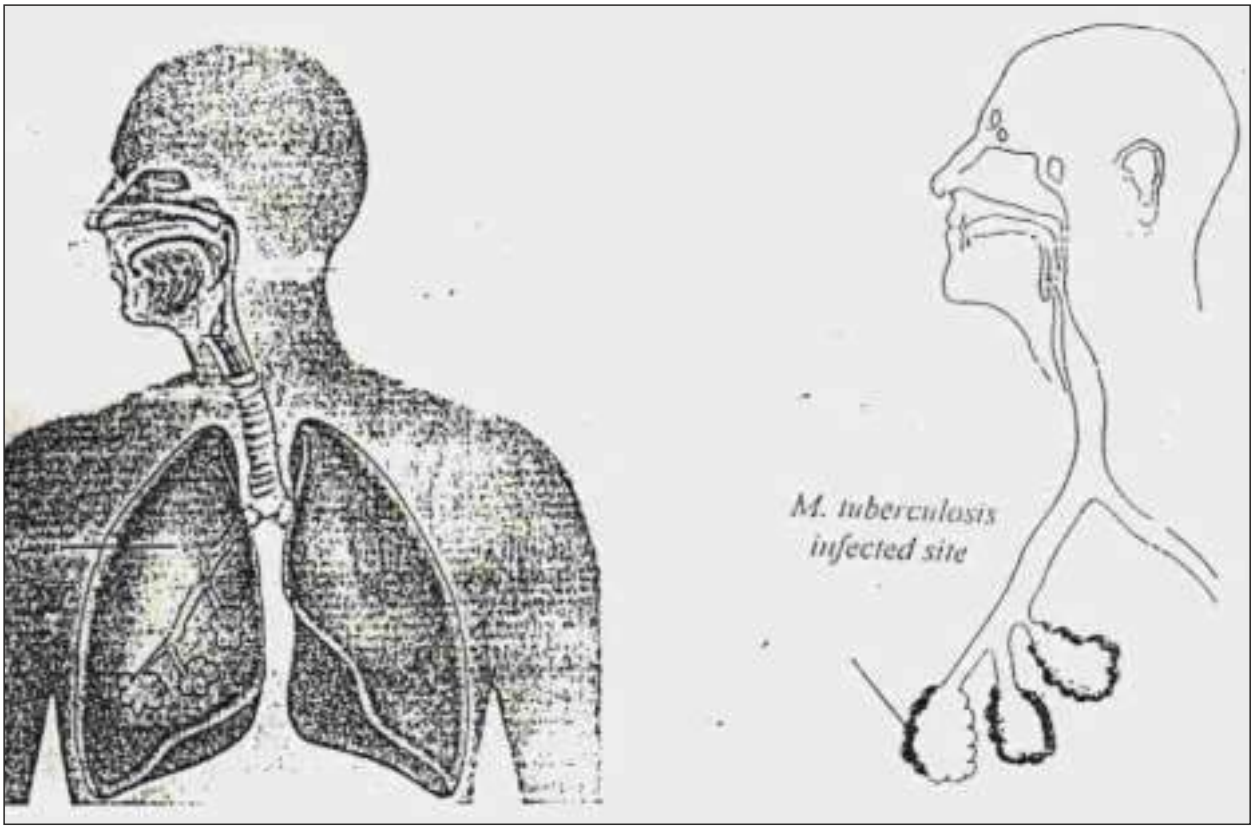


Fig 10. 1

Signs and symptoms:

- Chronic cough for more than 3 months.
- Loss of weight.
- Loss of appetite.
- Slight fever and sweating at night.
- Chest pain.
- Sometimes difficulty to breathe.
- Sometimes cough sputum with blood.

Diagnosis:

- To confirm an active case of pulmonary TB, microscopic examination of SPUTUM should be done to see whether it contains TB bacilli (Acid Fast Bacilli = AFB). Acid Fast Bacilli are small and irregular shaped bacilli.
- The request test for the laboratory exam is called " Sputum for AFB".
- If this test is not conclusive, a chest x-ray in the hospital can confirm whether there are any small holes in the lungs.

Treatment:

- TB bacilli very easily become DRUG RESISTANT; so many drugs are used at the same time.
- Treatment is long and must be taken on a regular basis, for at least 6 months.

11.1 SAFETY PRECAUTIONS

W.H.O (World Health Organization) has classified microorganisms into 4 groups according to the level of danger they represent to the laboratory technician and the community. Mycobacterium tuberculosis is classified in risk group III.

Risk group III is define as a group containing organisms that present a high risk to the laboratory worker.

In order to avoid being infected in the lab; follow the safety rules listed below.

I. GENERAL SAFETY RULES

- Eating in the lab is not permitted.
- Keep children out of the lab.
- The laboratory must be kept neat, clean and free of materials not useful to the work.
- A special place, separated if possible, should be kept exclusively for the smearing and fixing of sputum. This place should be well ventilated (but not windy) and with as much light as possible.
- This TB working area should have a bench with all the material required and a closed dustbin.

No material should leave this place.

Materials used in other departments should not be brought to this place.

- While working, only one lab technician should be in the room.
- The bench must be decontaminated every day after smearing with alcohol or with chlorine or bleach solution.
- The content of the bin must be disposed of daily and incinerated. Someone has to be responsible for that.
- Specimen should be collected in a clean (need not to be sterile), dry, wide-necked and leak proof container. Plastic bags are not recommended.

II SAFETY RULES APPLYING TO THE WORKER

- Wearing a **"duck" mask with micro-pore filtration < 140 mm** is recommended. Surgical masks and cotton masks give a false impression of safety.
- Wear gloves while smearing and fixing. Remove and dispose of them before leaving the working area.
- Always wash your hands with soap when work is completed.
- **Follow exactly the technical procedure for smearing and fixing.**

11.2 SPUTUM COLLECTION

I PREPARE THE SPUTUM CONTAINER

Give clean containers to the patient.

On the side of container (not on the lid), the following information should be written (see figure 10. 2):

- The patient's name.
- The date of each sputum collection.
- The sputum number.

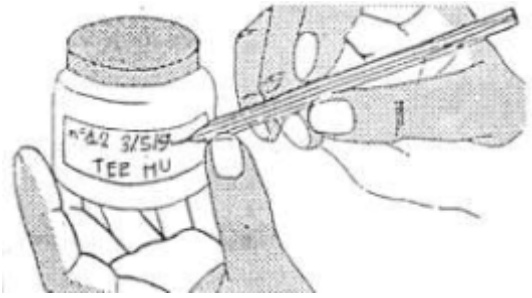


Fig 10. 2

II SPUTUM COLLECTION

This is the responsibility of medics, nurses or lab technicians: patients should be carefully instructed about sputum collection.

This MUST NOT be done in the lab. It should be done in special well-aerated room or outside with nobody around.

Tell the patient to spit in the morning:

1) The patient should be standing if possible.
2) Before eating anything, just when he gets up, he should wash his mouth by water first. This will avoid the bacteria and cells from the mouth.

3) Take a very deep breath, filling his lung.

4) He should empty his lung in one breath; cough as hard and deeply as he can.

5) Spit into the container.

The patient must spit every morning during 3 consecutive days.

III SPUTUM SPECIMEN CONTROL

The patients should bring the sample with the request from correctly filled to the laboratory technician.

You must check the quality and quantity of the sputum specimen.

The quality of the sputum:

Good sputum contains:

- Particle of pus (white, yellow or green), thread of fibrin, thick mucus and occasionally brownish streaks of blood.

Bad sputum is a liquid secretion and transparent (only saliva) coming from the mouth and Should not be processed.

The quantity of the sputum:

The quantity of the sputum should be at least 2 ml.

11.3 SPUTUM SMEARING

Materials (see figure 10. 3):

Fig 10. 3

- New frosted-end slides
- Pencil
- Examination gloves
- "Duck" mask
- Bamboo sticks
- Wide mouth pot with sand immersed in alcohol
- Spirit lamp, matches
- Dustbin

FOLLOW THE ORDER FOR SAFETY REASON

Method:

1. Clean the slides
2. Write on each slide (see fig 10. 4):
   - The patient's name
   - The number of the sputum
   - The date written on the container

Fig 10. 4

3. Put the gloves and mask on.

4. Light the flame of the spirit lamp.

FOR THE NEXT STEPS, ALWAYS KEEP THE LIGHTED LAMP BETWEEN YOU AND THE SPECIMEN. (see figure 10. 5)

Fig 10. 5: How to work safely

5. Carefully open the specimen container POINTING AWAY FROM YOU (see figure 10. 5).

6. With two bamboo sticks take a solid part of sputum (pus) and put it on the slide.

7. With the point of the sticks, smear the sputum as regularly as possible, with a circular movement. The smear should be in an oval shape of about 3 cm across. (see figure 10. 6).

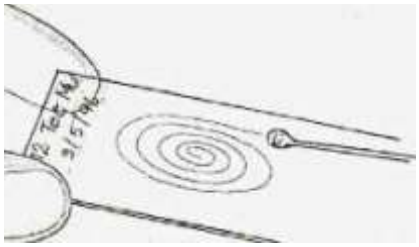


Fig 10. 6

8. Rub the bamboo sticks into the wide-mouth pot containing sand and alcohol in order to remove all of the sputum particles from the sticks.

9. Burn the sticks up to the middle. Throw them in the dustbin.

10. Close the specimen container. Do not discard yet; if anything happens, you may still need the specimen.

11. Let the smear air-dry in a safe place protected from flies, ants and dust.

12. Remove your mask and gloves, and dispose of in the bin.

13. Wash your hands.

14. When the smear is completely dry, fix it by passing the slide over the flame 3 times (smear on the upper side). Don't burn it. It must be possible to lay the slide on the back of your hand without feeling uncomfortably hot. Allow the smear to cool down before staining.

11.4 AFB STAIN OF SPUTUM SMEAR

There are 2 methods for staining a sputum smear for AFB in order to read the smear with the ordinary microscope:

I) Cold staining method

II) Hot staining method

STAINING METHOD:

1. Stain with carbol fuchsin.

5 minutes

I) Cold staining method:

Cover the cool side completely with the filtered, undiluted carbol fuchsin stain and leave it for 5 minutes.(see figure 10. 7)

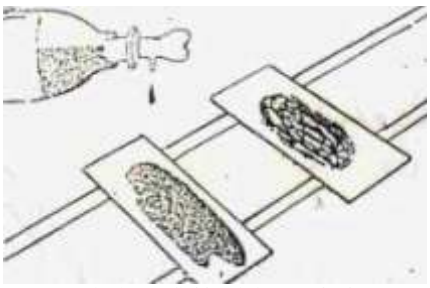


Fig 10. 7: Cold staining method.

II) Hot staining method (see figure 10. 8):

With this method, concentration of carbol fuchsin solution used is lower than the one for the cold method.

- Cover the smear completely with filtered, undiluted carbol fuchsin.
- Heat under the slide with a small flame unit vapour just begins to come off, then take the flame away. Do not boil the stain.
- Allow the stain to remain on slide for 5 minutes.

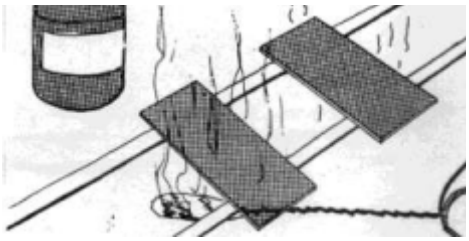


Fig 10. 8: Hot staining method

CARBOL FUCHSIN STAINS ALL THE ORGANISMS IN THE SPUTUM RED

2. **Wash the slide gently with WATER and drain it well.**
3. **Decolourise with 3% acid-alcohol.** 2 minutes
   - Cover the slides completely with 3% acid alcohol.
   - Leave it for 2 minutes or until no more colour appears in the washing (see figure10. 10).

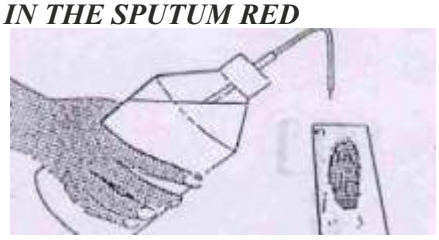


Fig 10. 9

Note:

3% acid-alcohol is made by carefully mixing 3 ml of hydrochloric acid into 97 ml of ethanol.

DO NOT MOUTH PIPETTE THESE SOLUTIONS!

ACID-ALCOHOL WILL REMOVE THE COLOUR FROM ALL ORGANISMS EXCEPT ACID – FAST BACILLI (AFB)

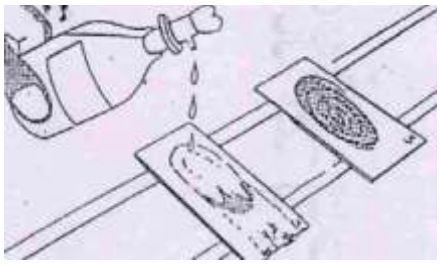


Fig 10.10

4. **Wash gently with WATER and drain well.**
5. **Stain with methylene blue**. 30 seconds

   Cover the slide completely with filtered undiluted methylene blue for 30 seconds.

METHYLENE BLUE STAINS ALL THE ORGANISMS DECOLOURISED BY THE 3% ACID-ALCOHOL IN BLUE.

THE ACID – FAST BACILLI REMAIN RED.

6. Wash gently with WATER and drain well.
7. Dry the slide in the air.

11.5 TB SLIDE EXAMINATION

I) Characteristic of tubercle bacilli from AFB stain.

- Red on a blue background.
- Straight or slightly curved.
- Quite short and thin.
- Often seen with red granules or spots on the bacilli, because they are often not uniformly stained.
- They can appear singly, arranged in small groups of 3-10 bacilli close together and in branches (like Chinese letters) or in big packs (less frequent). See figure 10. 11

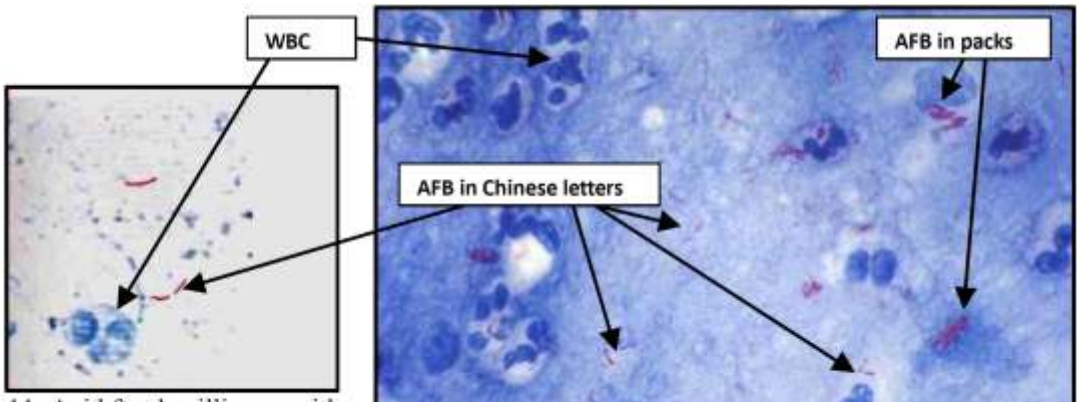


Fig 10. 11: Acid fast bacilli seen with X100 objective after staining.

II) Microscopic examination for AFB smear

The examination of each sputum smear should be done in a systematic way as follow:

1. Use ×100 objective (with oil immersion).
2. Start looking from the upper edge of the smear.
3. Examine the smear for AFB, following the pattern of movement shown in the figure bellow, one by one field systematically.
4. Continue the examination for approximately 300 fields to determine whether the smear is positive or negative for AFB. This should take at least 15 minutes.
5. If doubtful diagnosis, more fields should be examined.

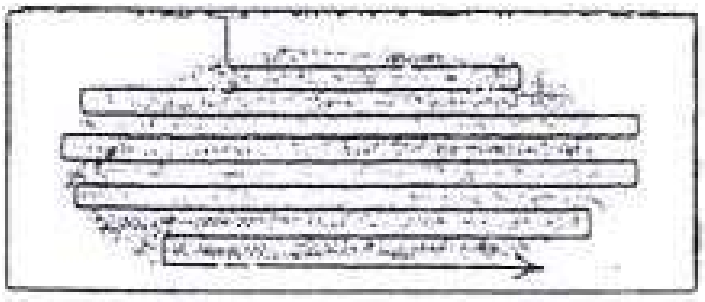


Fig 10. 12: How to examine an AFB smear.

11.6 TB RECORD

1) TB REGISTER BOOK

All the follow data should be written in the book:

- The patient's number
- The date
- The patient's name
- Sex
- Age
- Address
- TB results
- Lab technician signature

2) TB RESULTS

*** NEGATIVE SLIDE**

Examine the whole smear thoroughly at least 15 minutes

*** POSITIVE SLIDE**

If AFB is seen on the sputum smear, the TB result must be registered as follows:

NUMBER OF AFB FOUND:	**REPORT**
0 bacilli / 300 fields	NO AFB SEEN
1-2 bacilli / 300 fields	AFB RARE AFB
1-9 bacilli / 100 fields	+
1-9 bacilli / 10 fields 1-9 bacilli / field	AFB ++ AFB
>1-9 bacilli / field	+++ AFB

CHAPTER - 12

HEPATITIS B

Hepatitis means the infection of the liver.

Hepatitis is caused by a VIRUS called Hepatitis virus. There are many types of Hepatitis viruses e.g. Hepatitis A or Hep-A, Hepatitis B or Hep-B, Hep-C and Hep-E; which are transmitted by different ways, and present different stage of illness.

We are most concerned with Hep-B, which is transmitted by blood and secretions the same ways as HIV.

SIGNS AND SYMPTOMS:

Abdominal pain, jaundice, weight loss and weakness

Chronic infection of Hepatitis B virus shows high possibility of liver cancer.

Some people are infected with this virus in their body without any illness or symptoms present but they still can transmit the virus to others. These people are called carriers.

PREVENTION:

Same way as for HIV infection.

LABORATORY EXAMINATION FOR HEPATITIS B INFECTION

We test for Hep-BsAg or Hepatitis B surface antigen, which is a kind of protein on the surface of this virus. This antigen can be found in the blood several weeks before any signs of illness and then for months after. In some people the antigen is found for the rest of their life.

HEP-BSAG TESTS

There are different tests being used in the field but all use the same principle to find the antigen. Blood sample is needed for the testing.

Hepatitis B surface antigen test

Hepatitis B virus (HBV) is a different virus from other hepatitis viruses like Hepatitis A, Hepatitis C, Hepatitis D, and Hepatitis E due to having DNA.

It affects the liver and causes acute and chronic infection. HBsAg stands for Hepatitis B surface antigen. HBsAg test can be performed in both rapid and ELISA methods. The Rapid tests are available in cassette and strips method both. The test is intended to be used as an aid in the recognition and diagnosis of acute infections and chronic infections carriers of the Hepatitis B Virus (HBV).

Procedure: follow instructions given in the kit insert.

CHAPTER - 13

DENGUE

It is Vector born disease caused by Aedes Mosquitos.

Dengue fever is break bone fever characterized by severe fever, headache, myalgia, arthralgia nausea vomiting eye pain rashes with these complained

Diagnosis

It is RNA virus, molecularly detect within 5 days.

It can be detect by direct RNA virus,

It can also be by NS1 antigen by ELISA technique.

Detect by ELISA/ RT PCR IgM specific dengue virus antibody.

Test for Dengue- Rapid

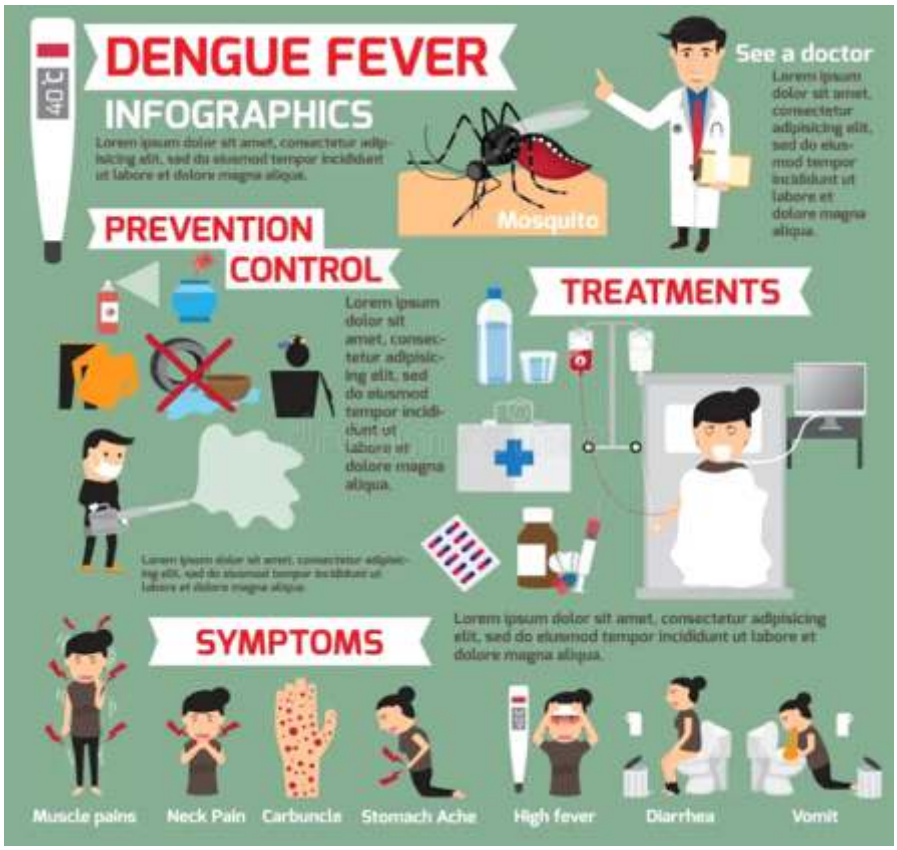
- NS1 tests detect the non-structural protein NS1 of dengue virus. This protein is secreted into the blood during dengue infection.
- NS1 tests have been developed for use in serum. Most of these tests use synthetically labeled antibodies to detect dengue NS1 protein.
- NS1 tests can be as sensitive as molecular tests during the first 0-7 days of symptoms. After day 7, NS1 tests are not recommended.
- A positive NS1 test result is indicative of a dengue infection but does not provide serotype information. Knowing the serotype of the infecting virus is not necessary for patient care; however, if serotype information is needed for surveillance purposes, the sample should be tested by NAT.
- Though studies show that NS1 can be found in whole blood or plasma, most NS1 tests have been developed and evaluated in serum samples. While combined testing with a NS1 and IgM antibody test can usually provide a diagnostic result during the first 1-7 days of
- illness, a second, convalescent phase specimen should be obtained and tested for IgM when both antigen and antibody tests are negative.

Procedure: follow instructions given in the kit insert.

Molecular tests for dengue

- For symptomatic persons with dengue virus infection, dengue virus RNA can usually be detected by molecular tests for the first 1-7 days in the course of illness.
- Specimen type- Serum, plasma, whole blood, cerebrospinal fluid
- Commercial diagnostic kits and clinical laboratory NAATs, including the CDC DENV-1-4 multiplex assay, are available.
- A positive NAAT result confirms dengue virus infection.
- A negative NAAT result does not rule out infection. People with NAAT negative results should be tested for the presence of IgM antibodies against dengue virus to determine possible recent dengue exposure.

Serological test for dengue



- Dengue virus-specific IgM and neutralizing antibodies typically develop toward the end of the first week of illness.
- IgM levels are variable, but generally are positive starting 4-5 days after onset of symptoms and continuing for approximately 12 weeks post symptom onset but may persist longer.
- Specimen type- serum, CSF
- The MAC-ELISA (IgM Antibody Capture Enzyme-Linked Immunosorbent Assay) is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus antigens. The antigens used for this assay are derived from the envelope proteins of the four dengue virus serotypes (DENV-1-4).
- Positive IgM: Patients with a positive IgM test result are classified as presumptive, recent dengue virus infections.
- Patients with negative IgM results before day 8 of illness and absent or negative NAAT or
- NS1 results are considered unconfirmed cases. For these cases, a second sample should be obtained after day 7 of symptoms for additional serologic testing.
- Due to cross-reaction with other flaviviruses and possible nonspecific reactivity, results may be difficult to interpret.

Recommended test for dengue

Diagnostic test	≤7 DaysAfter Symp tom Onset	>7 DaysPost Symptom Onset	Specimen type
MolecularTests	Yes	No	Serum, plasma, whole blood, ce- rebrospinalfluid*
Dengue Virus Anti- gen Détec- tion (NS1)	Yes	No	Serum
SerologicTests	Yes	Yes	Serum, ce-rebrospinalfluid*
Tissue Tests	Yes	Yes	Fixed tissues

CHAPTER - 14

CHIKUNGUNIA

It is Vector Born disease.

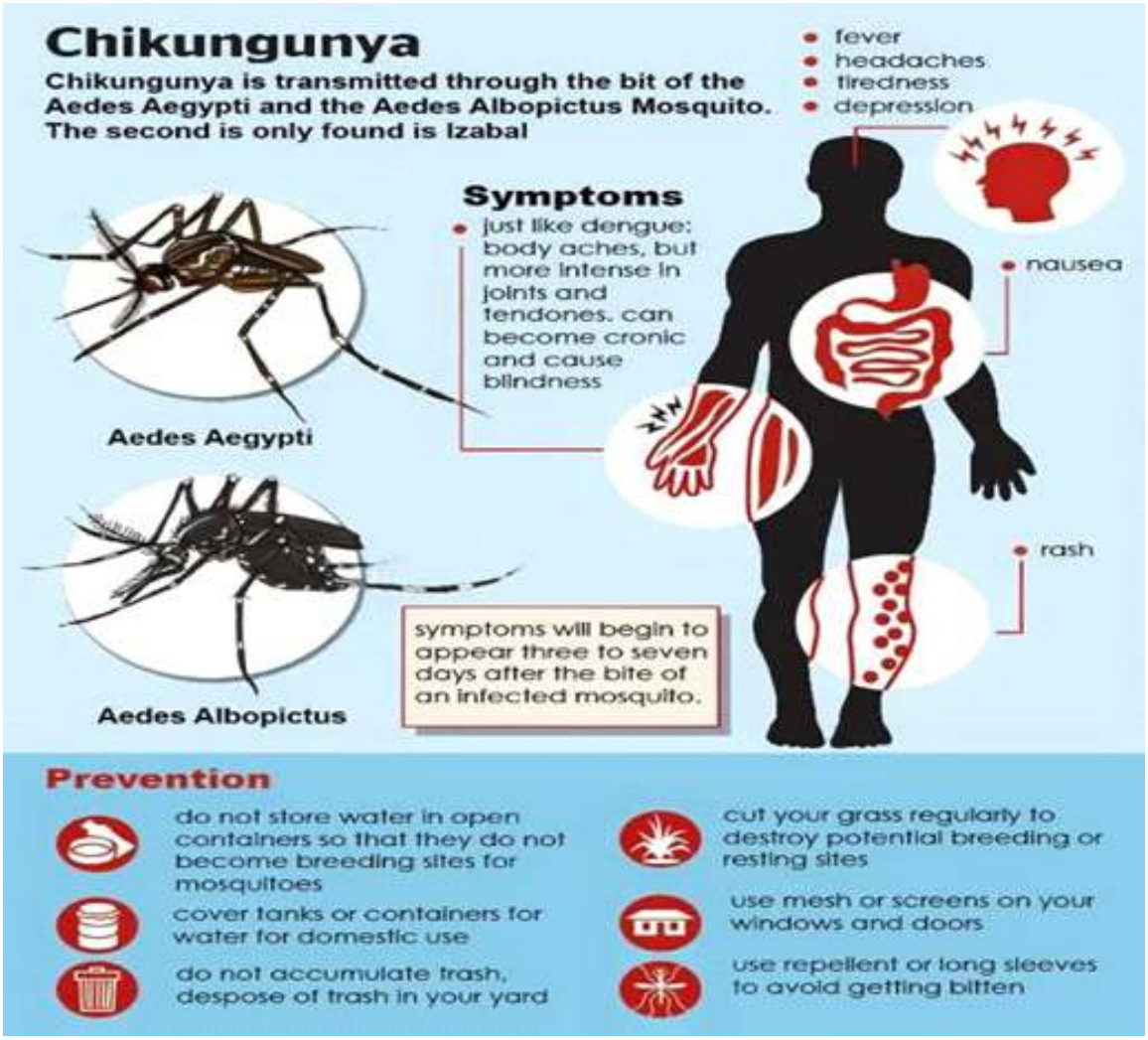
It is caused by Aedes Mosquitos..

Features found acute onset of fever with polyarthralgia means several joints are involved bilateral symmetrical and my severe and crippling other features may be common as other viral infections like headache nausea vomiting conjunctivitis, myalgia arthralgia oral mucosa and skin rashes.

Diagnosis-

Detection of direct RNA Virus with viral culture within 8 days of illness.

Detection of virus specific IgM antibodies after 7 days of illness by ELISA/RT PCR



CHAPTER - 15

JAPANESE ENCEPHALITIS

It is Ist time dected in Japan

It is also called brain fever

It is vector born disease

It is caused by Culex Mosquitos

This virus occurs along the Orient, from Korea and Japan in the north to India and Malaysia in the south.

The disease has been recognized in Japan since 1871

The virus was first isolated in Japan during an epidemic in 1935.

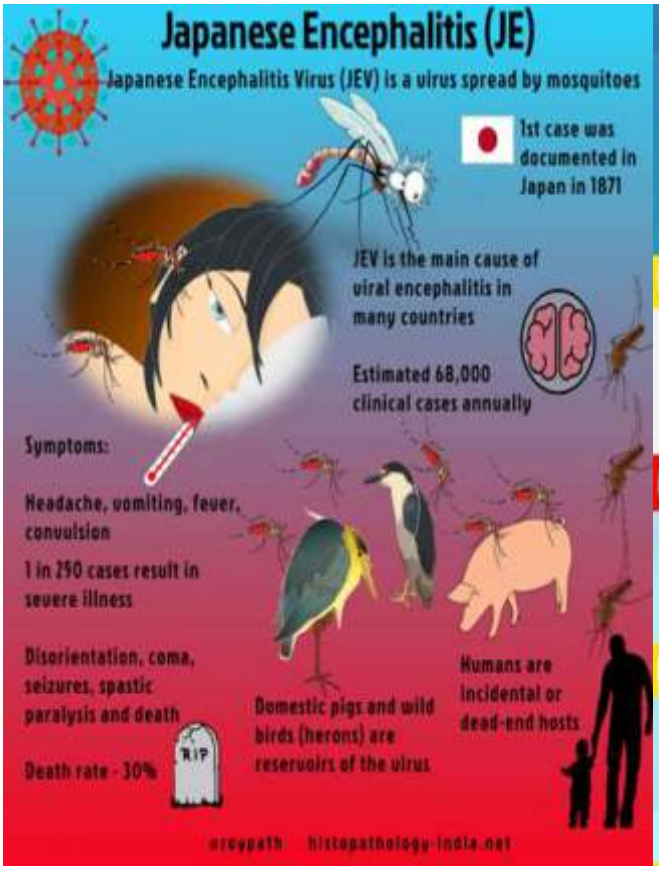
It is characterized by high grade fever, headache, vomiting and associated with neurological symptoms like convulsion seizures, altered sensorium, unconscious neurological deficit, breathlessness etc.

Laboratory Diagnosis-

Detection of Viral RNA by RT PCR in CSF early in illness.

Detection of viral specific IgM antibodies in CSF in illness

Detection of Viral specific IgM antibodies in Serum in latter CSF persists for 6 Month.



CHAPTER - 16

HIV and AIDS

- HIV stands for Human Immunodeficiency Virus which attaches to the White Blood Cells in the body and slowly kills them
- Acquired Immunodeficiency Syndrome refers to a disease that is :

  A Acquired (not inherited but contracted after birth)

  I Immune (weakens the immune system)

  D Deficiency (of certain white blood cells –Helper T4 lymphocytes in the immune system)

  S Syndrome (a group of symptoms or illnesses as a result of HIV infection)
- The difference between HIV and AIDS are that
- HIV is a virus and AIDS is a disease
- HIV infection is merely the presence of virus in the body
- AIDS is deficiency in the body's defence mechanism or immune system due to the presence of HIV
- HIV infection leads to AIDS, depending on the body's defence mechanism
- There are two types of HIV : HIV-1 and HIV-2. In India HIV -1 (Subtype C) is predominant.

Structure of HIV

- HIV virus is approximately 100 nm in diameter
- It has a lipid envelope, in which glycoprotein gp41 is embedded.
- Gp36 in case of HIV 2.
- Glycoprotein gp120 is attached.
- Inside envelope is a nucleocapsid (p17) which surrounds a central core of protein, p24.
- Within this core, are two copies of single-stranded RNA (the virus genome).
- The virus also contains three enzymes –
- Reverse transcriptase
- Integrase and
- Protease

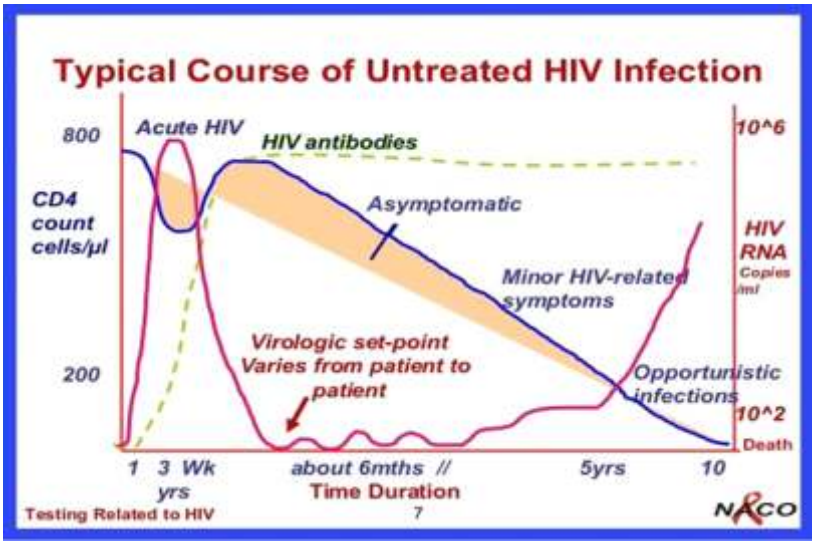
Spread of HIV

Not Spread of HIV

Work Precautions-

- It is stable for several hours at a pH between 3 and 10
- The methods used for sterilization and disinfection to kill the virus include autoclaving at 121°C at 15psi pressure for 20 minutes, use of dry heat, boiling, use of 1% Sodium hypochlorite/Ethanol/ Povidone iodine (PVI) or Glutaraldehyde (activated)
- Treatment of lyophilised HIV preparations at 100 °C (dry heat) for 10 min inactivates HIV completely
- At lower temperatures HIV is relatively stable: t/2 is at 20 °C approximately 9 h, at 4 °C several months and below −70 °C indefinitely.
- Half-Life Time in Blood and Plasma at body temperature of HIV is approximately 2 days and at 4 °C approximately 1 month.

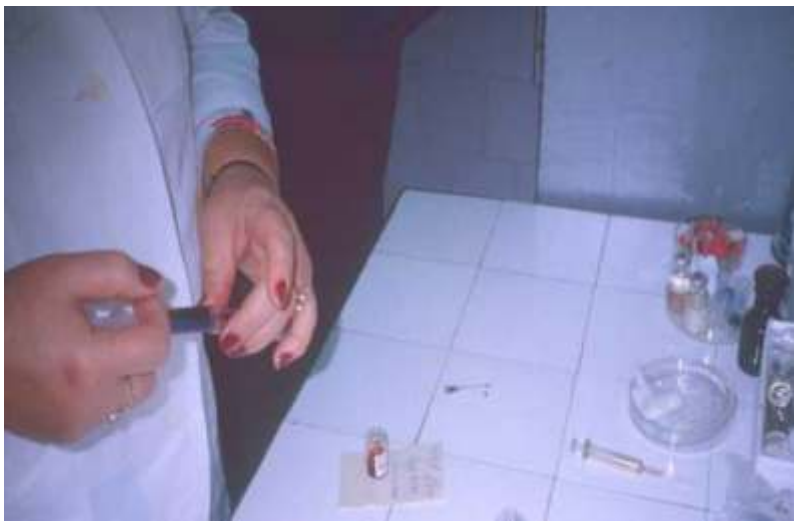
HIV 1 Infection Lab Markers for Diagnosis and monitoring-



WHO staging system for HIV infection in adult and adolescent after 13 Yrs age-

WHO staging system is categorized into four stages based on the clinical signs and symptoms

Detection of HIV Infection and Role of ART



- ❖ Only way to diagnose HIV infection is by laboratory testing for specific antibodies and/or the structural components of HIV
- ❖ Standard antiretroviral therapy (ART) consists of the combination of antiretroviral (ARV) drugs to maximally suppress the HIV virus and stop the progression of HIV disease.
- ❖ ART drugs act on various stages of replication of the virus in the body and interrupt the process of viral replication.
- ❖ In the absence of ART, CD4 cell count falls, PLHIV's immunity is compromised and they develop opportunistic infections and progress to the state of AIDS

Safety

- Implement activities related to personal safety
- Use standard precautions in laboratory setup
- Manage biomedical waste as per latest rules
- Understand PEP and its requirement

Identify the errors

Universal Work Precautions-

- Definition: Protective measures to be practiced by all health care workers at all times while providing professional services
- Mainly directed at handling blood, other body fluids and tissues to minimize the potential hazardous risk of pathogens like HIV/ HBV transmission

Hand Wash -

Clothing-

- ❖ Hand hygiene
- ❖ Use of Personal Protective Equipment
- ❖ Careful handling of sharps
- ❖ Safe techniques
- ❖ Disinfection
- ❖ Disposal of disposables/re-usable material
- ❖ Adherence to correct hospital sterilization and disinfection protocols
- ❖ Immunization against HBV
- ❖ BMWM

Dos	Don,ts
1. Pass syringes/needles in a tray	Never pass syringe or needle directly to next person
2.Remove cap of needle near the site of use .	Do not bend/break used needles with hands.
3,Pick up open needle from tray with a pair of forceps .	Never test the fineness of needles tip before use with bare or gloved hand
4.Destroy syringes by cutting the hub 5,Dispose of needles in a puncture proof container	Never pick up an open needle by hand

HIV (Antibodies to HIV 1&2)

- HIV is a lentivirus that infects and destroys cells in the immune system. Lentiviruses are in turn part of a larger group of viruses known as retroviruses. The name 'lentivirus' means 'slow virus.'
- HIV-1 is the most prevalent type throughout the world. HIV-2 has limited geographic distribution. HIV-1 is closely related to Simian Immunodeficiency Virus (SIV), prevalent in populations of wild chimpanzees in West Central Africa.
- The strips/cards incorporate both the antigen and signal reagent into the nitrocellulose strip. The specimen (usually followed by a buffer) is applied to the absorbent pad on the kit.
- The test device is incorporated with distinct bands of purified gp120 and gp41 synthetic peptides, specific to HIV-1 at test region '1' and gp36 synthetic peptide specific to HIV-2 at test region '2.' The third band incorporated at region 'C,' corresponds to the assay performance control. If present, antibodies to HIV-1 and/or 2 are captured by the respective antigens.
- After washing with a buffer, Protein A conjugated reagent is added to reveal the presence/ absence of bound antibodies.

Procedure: Follow the instructions and storage conditions given on the card/ test kit.

OTHER TESTS

Typhoid testing methods

(widal test quantitative and semiquantitative, Rapid test of typhoid testing)

- Widal test is a tube agglutination test employed in the serological diagnosis of enteric fever. The test is named after Georges Fernand Isidore Widal, a French physician and bacteriologist.
- **Principle:** Patients' suffering from enteric fever would possess antibodies in their sera which can react and agglutinate serial doubling dilutions of killed, colored Salmonella antigens in a tube agglutination test.
- **Requirements**: Widal rack, round-bottomed Felix tubes, conical-bottomed Dreyer's tubes, water bath, doubly diluted patient serum in three-four rows, Killed colored suspensions of S. typhi O antigen, S. typhi H antigen, S. paratyphi AH antigen and optionally S. paratyphi BH antigen

Figure 65: Reagent (Suspension Of S Typhi)
Source: https://images.app.goo.gl/Lwo5JhjSTkdevvvr9

Specimen collection procedure

- Don't use hemolysis sample.
- Don't heat or inactivate the serum.
- Freshly collected specimen in preferable, store at 2-8^{0}C up to 72 hrs in case of delay testing
- It is preferable to test two specimens of a Sera at an interval of 7 to 10 days to demonstrate a rising antibody titer.
- Salmonella antibodies start appearing in serum at the end of the first week and rise sharply during the third week of endemic fever.

In acute typhoid fever O agglutination can usually be detected 6-8 days after the onset of fever and H agglutination after 10-12 days

Procedure

- Slide method- Qualitative
- Reaction circle- Semi quantitative
- Tube method- Quantitative

Slide method (Quantitative)

1. Place one drop of positive control on one reaction circle of test card/ on slide
2. Pipette one drop normal saline on the next circle for negative control.
3. Pipette one drop of patient serum on to remaining 4 reaction circle.
4. Add one drop of H suspension on 1st and 2nd reaction circle (on pos and neg control slide) Add one drop each of O, H, AH and BH antigens to the remaining four reaction circles.
5. Mix content of each circle uniformly over the entire circle with separate mixing sticks. Rotate the slide, gently back and forth and observe for agglutination within one minute.

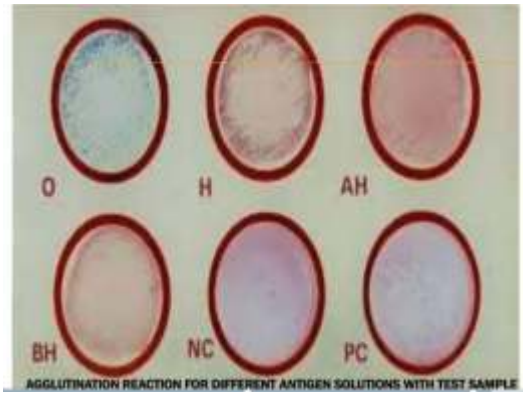


Figure 66: Agglutination Reaction For Different Antigen Solution With Sample
Source: https://images.app.goo.gl/Lwo5JhjSTkdevvvr9

Semi-quantitative method

Serum volume	Titer
80µl	1:20
40µl	1:40
20µl	1:80
10µl	1:160
5µl	1:320

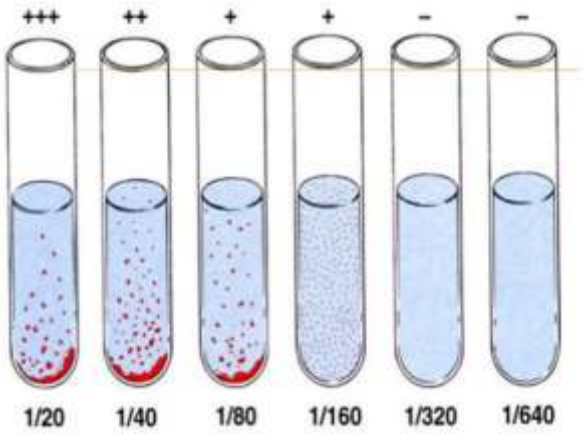
1. Pipette 5, 10, 20, 40, 80µl of the test sample on the reaction circles.
2. Add a drop of antigen to each circle which showed agglutination with test sample in screening method.
3. Mix the content using separate mixing sticks.
4. Rock the slides gently back and forth and observe agglutination.

Standard tube method (Quantitative)

1. Take 4 sets of 8 test tubes and level them 1-8 for O, H, AH and BH antibody.
2. Pipette 1.9 ml of isotonic saline into tube no 1 of all set
3. Add1 ml of isotonic solution of in all reaming tube (2-8)
4. Add 0.1 ml of tested serum sample to tube no 1 of each row.
5. Transfer 1 ml of diluted serum from tube no 1 to tube no 2. Repeat 1 ml dispensing 2 to 3 to 4 to 5 to 6 to 7 to discard (serial dilution)
6. Tube no 8 in all set serves as saline control. The dilution of serum sample archived in set as follows.
7. Tube number 1,2,3 4,5,6,7 (control)
8. Dilutions- 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280
9. To all tubes 1(to 8) of each set add one drop of the respective widal test antigen suspension (O, H, AH, BH) from reagent vials and mix well.
10. Cover the tubes and incubate at 37C overnight (aprox 18 hrs)
11. Dislodge the sedimented button gently and observe the agglutination.

Interpretation

- Agglutination indicates a positive test result.
- Significant title is >1:160 but varies from population to population.
- Titre of "O" antigen more than 1:160 indicates recent infection.
- Title of "H" antigen more than 1:160 indicates past infection or due to immunization.
- False positive result may be vaccination on previously infection with S. typhi.
- False negative may be due to antimicrobial treatment with blocks antibody response.



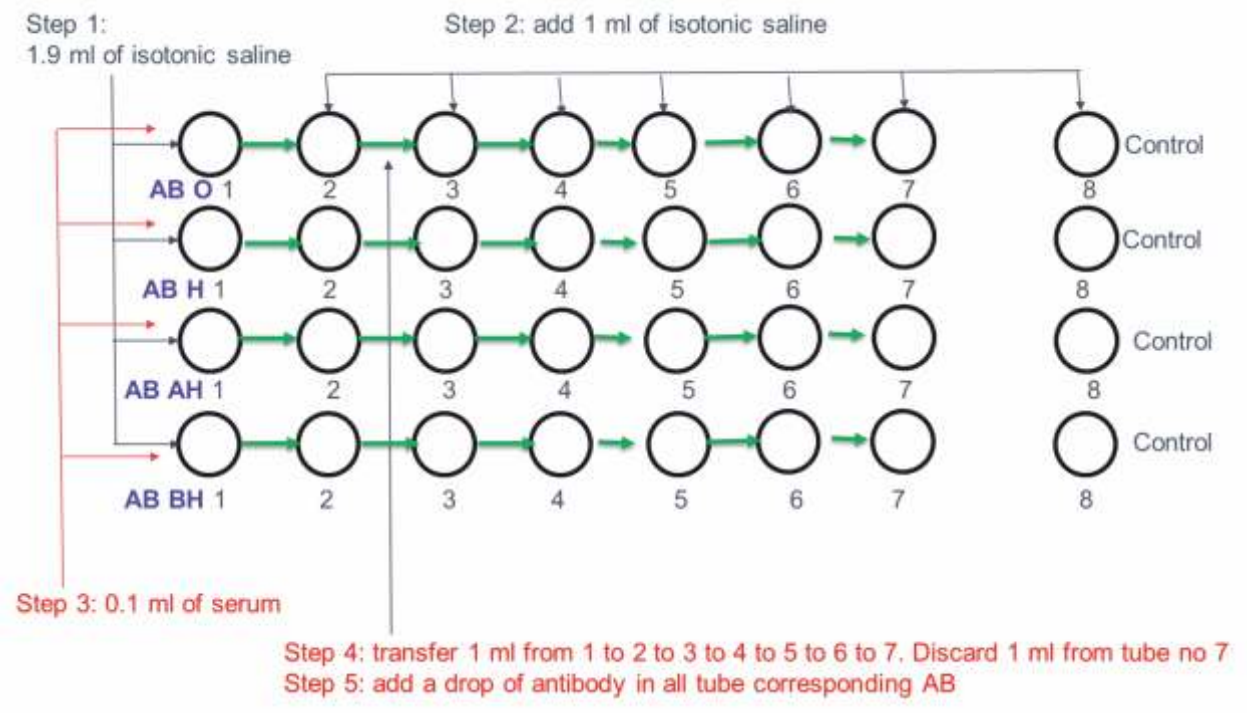


Figure 67: Quantitative Method (Tube Widal)

Bacterial meningitis

Diagnostic techniques for bacterial meningitis are:

1. CSF Culture- it will be covered in microbiology section.
2. CSF gram stain- will be covered in microbiology section.
3. Latex- agglutination
4. Raid test (RDT)

Latex Agglutination

- Several commercial kits are available for latex agglutination testing.
- For the best results, the supernatant of the centrifuged CSF specimen should be tested as soon as possible.
- If immediate testing is not possible, the CSF specimen can be refrigerated (between 2-8°C) for several hours, or frozen at -20°C for longer periods.
- It is imperative that the kits be kept refrigerated before use, but never frozen, especially in tropical climates as the kits deteriorate at high temperatures which may make the test results unreliable before the expiration date of the kit.

Procedure

1. Follow the manufacturer's instructions on the package insert for the specific latex kit being used. General instructions are listed below:
2. Centrifuge the CSF for 10-15 minutes at 1000 x g and collect the supernatant. Note: The sediment should be used for Gram stain and primary culture.
3. Heat the CSF supernatant to be used for the test at 100°C for 3 minutes.
4. Shake the latex reagents gently until homogenous.

5. Place one drop of each latex reagent on a disposable card provided in the kit or a ringed glass slide.
6. Add 30-50 µl of the supernatant of the CSF to each latex reagent.
7. Rotate by hand for 2-10 minutes. If available, mechanical rotation at 100 rpm is recommended- Avoid cross-contamination when mixing and dispensing reagents.
8. Examine the agglutination reactions under a bright light without magnification.
9. **Positive reaction:** agglutination (or visible clumping) of the latex particles and slight clearing of the suspension occurs within 2-10 minutes.

Figure 68: Bacterial Meningitis (Agglutination)
Source: https://images.app.goo.gl/gpWsmP- gdsZ5E91T27

10. **Negative reaction**: the suspension remains homogenous and slightly milky in appearance.

RDT for meningococcal meningitis

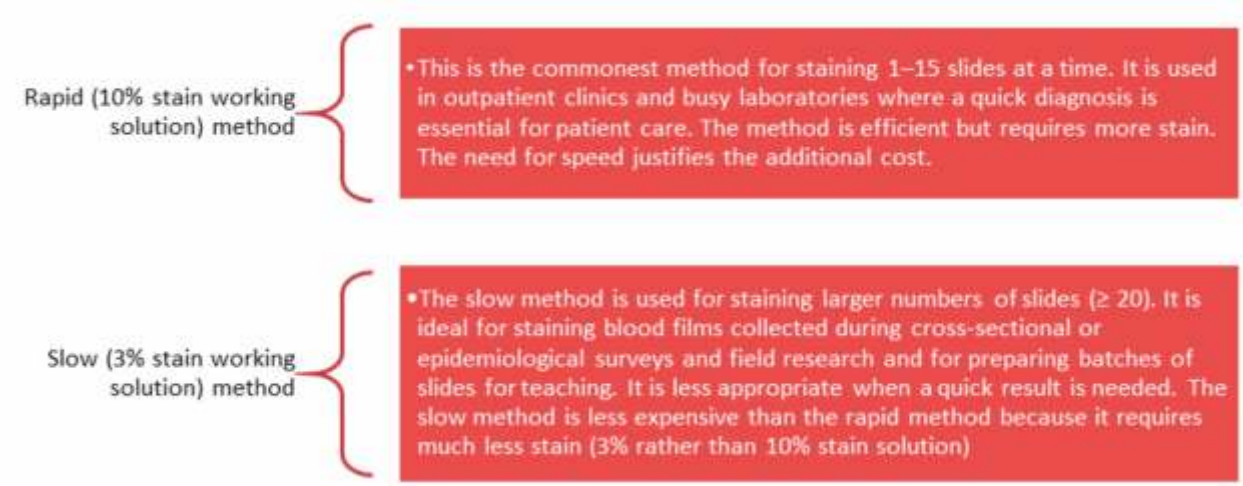
- RDTs have been developed for direct testing of CSF specimens without prior heat or centrifugation.
- The test is based on the principle of vertical flow immunochromatography in which gold particles and nitrocellulose membranes are coated with monoclonal antibodies to capture soluble serogroup-specific polysaccharide antigens in the CSF.
- The test consists of 2 duplex paper sticks (also called dipsticks), which together enable identification of four serogroups of N. meningitidis (A, C, W135, and Y). RDT1 tests for serogroups A and W135/Y and RDT2 tests for serogroups C and Y.
- RDTs can be produced in large quantities, are relatively inexpensive, and remain stable for weeks in hot weather if protected from humidity; therefore, they are practical for immediate testing of specimens obtained during adverse conditions.
- Initial evaluation using RDTs on stored CSF from patients in Neither showed correct identification of the meningococcal serogroup 97% of the time.
- However, more recent studies under field conditions have shown similar specificity, but much lower sensitivity of 70% (6) and, in contrast, similar sensitivity but much lower specificity.

Geisma Staining of Blood

film.

- A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species.
- The use of Giemsa stain is recommended and most reliable procedure for staining thick and thin blood films. Giemsa solution is composed of eosin and methylene blue (azure).
- The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue.
- The thin film is fixed with methanol.
- De-haemoglobinization of the thick film and staining take place at the same time.
- The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2.

Geisma staining methods.



Rapid (10%) Geisma staining procedure

1. Prepare 10% Giemsa working solution and place it in a small container.
2. Using a Pasteur pipette, fix the thin film by carefully dropping methanol onto the thin film only.
3. Let the blood film dry in air on a drying rack or tray.
4. Place slides for staining blood films face down on a curved staining tray or face up on a staining rack.
5. Pour stain slowly on or under the slide until the blood films are covered.
6. Set the timer to 8-10 minutes for the staining.
7. Gently flush all the stain from the slides by dropping clean water over it.
8. Allow the slides to air-dry.
9. Discard the remaining 10% Giemsa solution.

Slow (3%) Geisma staining protocol

1. Prepare a 3% Giemsa working solution and place it in a small container.
2. Fix only the thin film with methanol. Avoid contact between the thick film and methanol to avoid accidental fixation.
3. Allow the blood film to dry in air on a drying rack or tray
4. Place the slides back-to-back in a staining tray.
5. Pour stain slowly on the slides. Do not pour it directly onto the thick films.
6. Set the timer to 45-60 minutes and stain the blood film.
7. Gently pour clean water into the tray to float off the iridescent scum.
8. Gently pour off the remaining stain, and rinse with clean water.
9. Carefully remove the slides and allow them to dry.
10. Discard the remaining 3% Giemsa solution.

Pap smear

- To define the process of managing Cervical
- Cancer Screening.

- Cervical cancer screening is used to find changes in the cells of the cervix that could lead to cancer. Screening includes cervical cytology (also called the Pap test or Pap smear), testing for human papillomavirus (HPV), or both. Most women should have cervical cancer screening on a regular basis.
- Two methods are widely used for sampling,

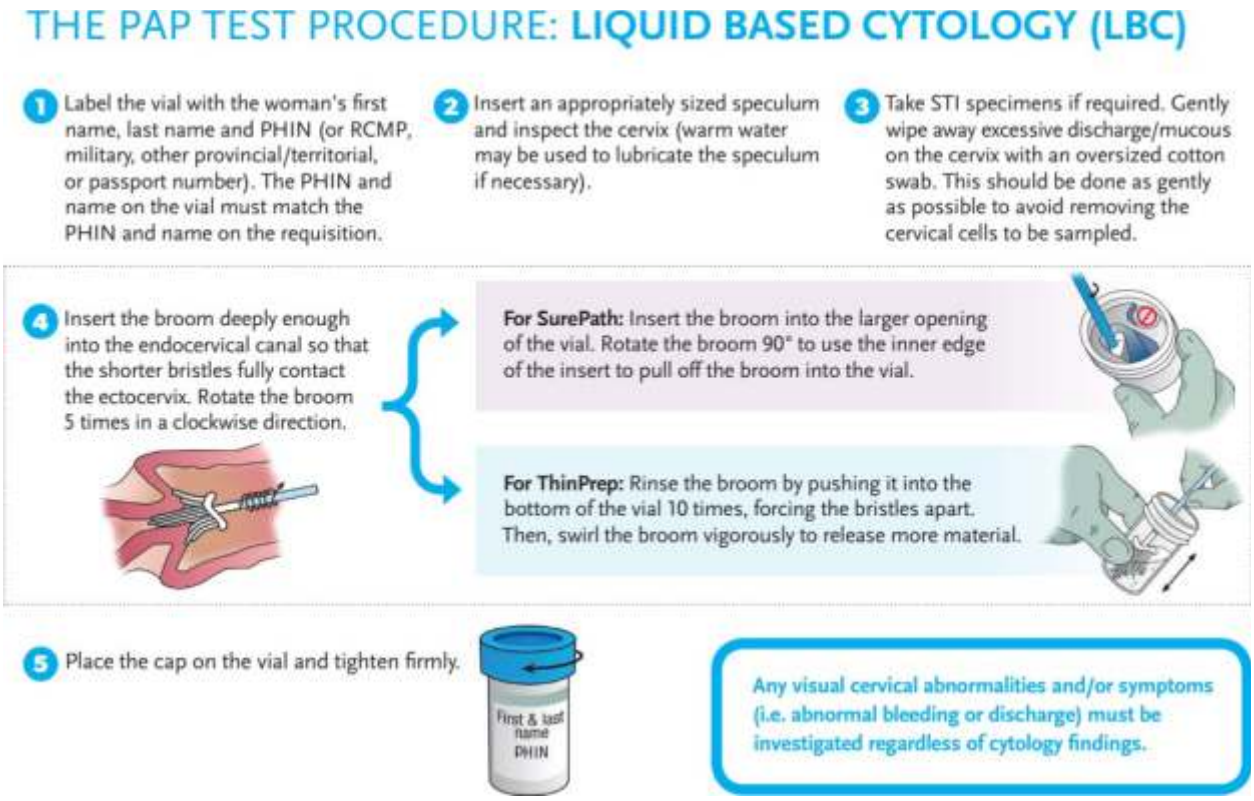


Figure 69: Lbc Sampling Method (Cancertalk_spring2015-Insert_sampling_technique.pdf)
Source: CancerTalk_Spring2015-Insert_Sampling_technique.pdf

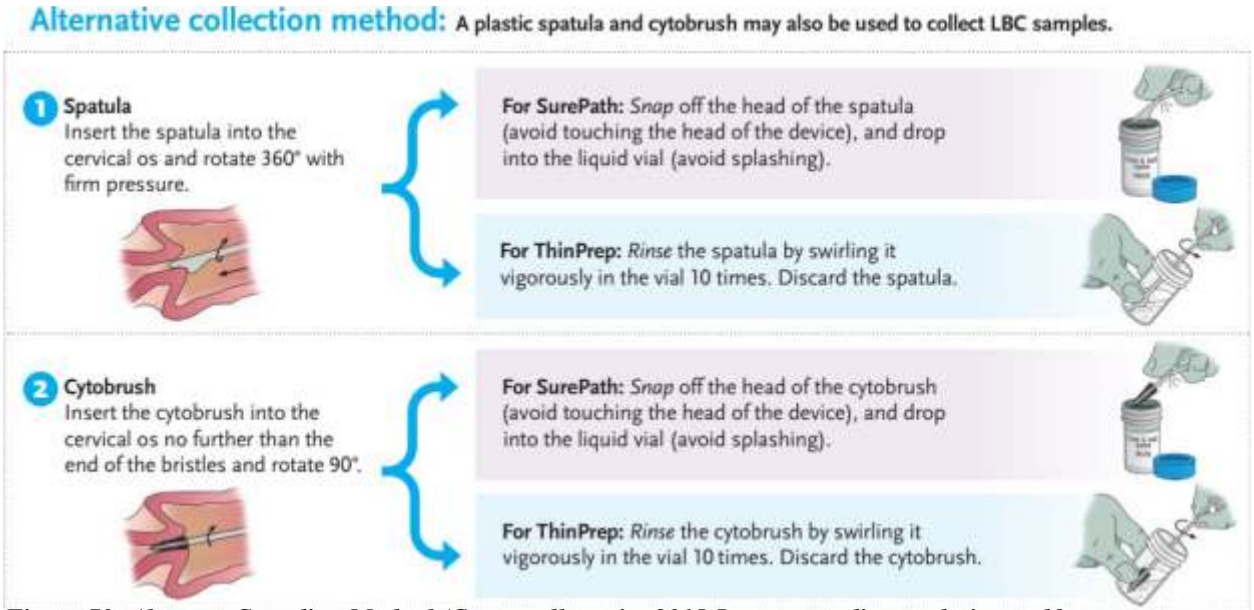


Figure 70: Alternate Sampling Method (Cancertalk_spring2015-Insert_sampling_technique.pdf)
REF: https://www.cancercare.mb.ca/export/sites/default/For-Health-Professionals/.galleries/files/publications- files/cancer-talk -files/CancerTalk_Spring2015-Insert_Sampling_technique.pdf source: CancerTalk_Spring2015-Insert_Sampling_technique.pdf

Cytology Staining method

1. The Papanicolaou stain is recommended for the staining of alcohol fixed cytology slides.
2. Romanowsky stains may also be used for wet fixed slides but are primarily applied to air-dried smears.
3. Special stains are used as per requirements.
4. Modified Ziehl Neilson (for acid fast bacilli), Gram staining (Bacteria), Mucicarmine (mucins), PAS (for glycogen, fungal wall, lipofuscin, etc), Oil red O (lipids), Perl's Prussian blue (iron), modified Fouchet's test (bilirubin), etc.
5. Immunocytochemistry is also being increasingly used in cytology specimens.
6. The universal stain for cytological preparations is the Papanicolaou stain. Harris' hematoxylin is the optimum nuclear stain and the combination of OG6 and EA50 give the subtle range of green, blue and pink hues to the cell cytoplasm.

Original Papanicolaou staining method.

1. Add 96% ethyl alcohol for 15 seconds.
2. Add 70% ethyl alcohol for 15 seconds.
3. Add 50% ethyl alcohol for 15 seconds.
4. Wash with Distilled water 15 seconds
5. Add Harris hematoxylin for 6 minutes.
6. Wash with distilled water 10 dips
7. Add Hydrochloric acid 0.5% solution, 1-2 quick dips
8. Wash with distilled water 15 seconds
9. Add a few dips in 0.1% ammoniated water. The smear turns to blue.
10. Add 50% ethyl alcohol for 15 seconds.
11. Add 70% ethyl alcohol for 15 seconds.
12. Add 96% ethyl alcohol 15 for seconds.
13. Add OG-6 (orange) for 2 minutes.
14. Add 96% ethyl alcohol 10 dips.
15. Add 96% ethyl alcohol 10 dips.
16. Add EA 50 eosin yellowish 3 minutes.
17. Add 96% ethyl alcohol (10 dips)
18. Add 100% ethyl alcohol (10 dips)
19. Add Xylene (10 dips)
20. Mount: in DPX using coverslip

Stain 1. Harris' hematoxylin	
Hematoxylin	5g
Ethanol	50 ml
Potassium alum	100g
Distilled water (50°C)	100 ml
Mercuric oxide	2-5 gm
Glacial acetic acid	40 ml

Stain 3. EA 50	
0.04 M light green SF	10 ml
0.3M eosin Y	20 ml
Phosphotungstic acid	2 gm
Alcohol	750 ml
Methanol	250 ml
Glacial acetic acid	20 ml

Result: The nuclei should appear blue/black. The cytoplasm (non-keratinizing squamous cells) – blue/ green. Keratinising cells- pink/orange.

MAY-GRÜNWALD GIEMSA STAIN

This is one of the common Romanwsky stains used in cytology.

- It is useful for studying cell morphology in air- dried smears.
- It is superior to Papanicolaou to study the cytoplasm, granules, vacuoles, basement membrane material etc.
- For nuclear staining Papanicolaou is superior

Giemsa solution	
Methanol	73 %
Glycerol	26%
Giemsa´s Azur-Eosin-Methylene blue	0.6%
Phosphate Buffer	
Potassium dihydrogen phosphate/ di-sodium hydrogen phosphate x 2H2O	67 mmol/l

Contents of the staining reagents	
May-Grünwald solution	0.2%
Methanol	99%
May-Grünwald´s eosin-methy-lene blue	0.2%

Preparation of working solutions (MAY-GRÜNWALD GIEMSA STAIN)

1. **Buffered water:** Dilute phosphate buffer with deionised or distilled water. 1:20, e.g. 30 ml phosphate buffer + 570 ml deionised or distilled water.
2. **Giemsa working solution**: Mix 84 ml of Giemsa solution into 516 ml of buffered water.
3. **May-Grünwald working solution**: Mix 360 ml of May-Grünwald solution into 240 ml of buffered water.

Storage: Giemsa solution, May-Grünwald solution: protected from light at 2-25°C. Unopened reagents may be used until the expiry date on the label.

Phosphate buffer: at 2-8°C. Unopened reagents may be used until the expiry date on the label.

Staining Procedure (MAY-GRÜNWALD GIEMSA STAIN)

1. Fix the air-dried smear specimen in methanol for 10 -20 minutes.
2. Stain with May-Grünwald working solution for 5 minutes.
3. Stain with Giemsa working solution for 12 minutes.
4. Wash with clean buffered water for 2, 5 and 2 minutes
5. Dry the slides in upright position at room temperature.
6. Mount the slides with a coverslip using DPX.

Ziehl-Neilsen staining procedure.

1. Place fixed slides on the staining rack in serial order, smeared side up.
2. Slides should be separated by a 1 cm gap and should never touch one another.
3. Cover slides individually with filtered Ziehl's carbol fuchsin working solution.
4. Heat slides from underneath with the flame of a Bunsen burner, an alcohol lamp or an alcohol- soaked cotton swab until vapour starts to rise.

Ziehl-Nielsen straining reagent

Reagents	
1. Carbol fuschin	
Basic Fuchsin	1 gm
Absolute alcohol	10 ml
Add the basic fuchsin to the alcohol in a 100 ml flask and mix, on a magnetic stirrer for 30 min- utes. Add 100ml of 5% aqueous phenol. Mix well.Filter and store in a brown glass bottle.	
2. Acidified methylene	
0.25% methylene blue in 1% acetic alcohol	
3. 0.5% Acid Alcohol	
Distiller water	700 ml
Absolute alcohol	300 ml
Hydrochloric acid	5 ml

Caution: Always add acid to water while diluting. Adding water in acid is dangerous.

5. Staining solution should never be allowed to boil. Do not allow the stain to dry.
6. Keep slides covered with hot, steaming carbol fuchsin for 5 minutes by re-flaming as needed.
7. Rinse slides gently with water to remove excess carbol fuchsin.
8. Drain off excess rinsing water from slides.
9. Sputum smears appear red in colour

Visual inspection with acetic acid

- Government of India is undertaking a population-based cancer screening of three common cancers in the country: oral, breast and cervical cancer.
- According to the operational guidelines, cervical cancer screening will be done using Visual Inspection with Acetic acid (VIA) by ANMs and staff nurses at the Primary Health Centre (PHC) level.
- detailed manual can find throw link https://www.wbhealth.gov.in/NCD/uploaded_files/ all_files/VIA_Module_NICPR.pdf

Fecal Occult Blood test

- The fecal occult blood test (FOBT) is a diagnostic test to assess occult blood in the stool (Occult means Hidden).
- This test has commonly been used for colorectal cancer screening.
- Colon cancer is one of the most prevalent cancers in both men and women worldwide, therefore, early detection is imperative.
- Newer screening methods, including the fecal immunochemical test (FIT), have been developed. FIT uses antibodies to discern blood in the stool.
- Specimen Collection
- This test is performed in either the inpatient or outpatient setting. In the inpatient setting, the stool is often obtained manually during a digital rect.
- In the outpatient setting, the patient typically obtains a stool sample at home and then submits it to a laboratory examination and placed onto heme occult testing cards.

Fecal Occult procedure- Rapid method

- The FOB One Step Fecal Occult Blood Test Strip (Feces) is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in feces.

Principal

- The FOB One Step Fecal Occult Blood Test Strip (Feces) is a qualitative, lateral flow immunoassay for the detection of human occult blood in feces.
- The membrane is precoated with anti- hemoglobin antibody on the test line region of the Strip. During testing, the specimen reacts with a particle coated with anti-hemoglobin antibody.
- The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin antibody on the membrane and generate a colored line.
- The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.
- Rapid tests are available in card and strip, kindly follow the manufacturers direction for testing.

RTI/STI

- Laboratory tests improve the diagnostic sensitivity and specificity of STI/RTI syndromes, and particularly in women, help to differentiate serious infections (e.g., cervicitis) from milder but more common infections (e.g. vaginitis).
- The tests also help in detection of infections in asymptomatic individuals.
- Laboratory diagnosis of STI/RTI includes three major equally important steps, i.e., collection of specimens, its transport and use of a sensitive and specific test.
- Laboratory procedures should include microscopic examination of fresh and stained specimens. The minimal laboratory tests recommended under the NACO programme are:
- RPR (Rapid Plasma Regain) test for syphilis testing (qualitative and quantitative) for STI/RTI attendees and ANC attendees.
- Wet-mount slide preparations for microscopic examination:
    - Normal saline slide preparation for detection of motile trichomonads.
    - KOH slide preparation for detection of Candida spores and pseudohyphae, and "Whiff test" for detection of amines indicative of bacterial vaginosis (Whiff test to be performed by the examining clinician)
- Determination of pH level of vaginal secretions (to be performed by the examining clinician). Gram stain of cervical/rectal specimen for white blood cell (WBC) and gram-negative intracellular diplococci.
- Gram stain of slides prepared from vaginal smears to diagnose bacterial vaginosis.

Vaginal pH:

- The pH of vaginal fluid should be measured using pH paper of appropriate range (3.8 to 6.0).
- The vaginal fluid sample is collected with a swab from the lateral and posterior fornices of the vagina and the swab is then touched directly on to the paper strip. Alternatively, the pH paper can be touched to the tip of the speculum after it has been withdrawn from the vagina.
- Care must be taken not to use any jelly (e.g. KY jelly) or disinfectant (e.g. savlon) before doing the pH test
- The normal vaginal pH is 4.0.
- In BV, the pH is generally elevated to more than 4.5. An elevated pH is also observed if the vaginal fluid is contaminated with menstrual blood, cervical mucus or semen, and in women with a T. vaginalis infection.
- In simple words it means that if the pH test is negative the result can be taken as it is but if it is positive one has to rule out other factors contaminating the sample such as menstrual blood, cervical mucus, semen or presence of T. vaginalis infection.

Clinical criteria for Bacterial Vaginosis (BV)

Collect specimen	· Note colour and consistency of discharge. · Take a specimen of discharge from the sidewalls or deep in the vagina where discharge pools (or use discharge remaining on speculum). · Touch pH paper to discharge on swab or speculum and note pH
Prepare slide	· Place specimen on a glass slide. Add a drop of 10% potassium hydroxide (KOH)and note for any fishy smell. · Make a wet smear with 0.9% normal saline, cover with cover slip and see under microscope for clue cells.

What to look for	The diagnosis of BV is based on the presence of at least 3 of the 4 following characteristics. a. Homogeneous white-grey discharge that sticks to the vaginal walls. b. Vaginal fluid pH>4.5 c. Release of fishy amine odor from the vaginal fluid when mixed with 10% potassium hydroxide (positive whiff test). d. "Clue cells" visible on microscopy on wet preparation
Important	· Look for evidence of other vaginal or cervical infections as multiple infections are common.

Wet mount microscopy

Collect specimen	Take a specimen of discharge with a spatula from the sidewalls or deep in the vaginawhere the discharge accumulates.
Prepare slide	Mix specimen with 1 or 2 drops of saline on a glass slide and cover with a cover slip.
What to look for	· Examine at 100X magnification and look for typical jerky movement of motile trichomonads (ovoid, globular, pear-shaped flagellated protozoan). · Examine at 400X magnification to look for yeast cells (round to ovoid cells with typical budding) and trichomonads. · To make identification of yeast cells easier in wet mount slides, mix the vaginalswab in another drop of saline and add a drop of 10% potassium hydroxide to dissolve other cells and note any fishy odor. · Presence of clue cells (squamous epithelial cells covered with many small coc-cobacillary organisms). Wet mount shows stippled granular cells without clearly defined edges because of the large numbers of adherent bacteria present and an apparent disintegration of the cells. (The adhering bacteria are predominantly G. vaginalis, sometimes mixed with anaerobes).
Important	· Look for evidence of other vaginal or cervical infections as multiple infections are common.

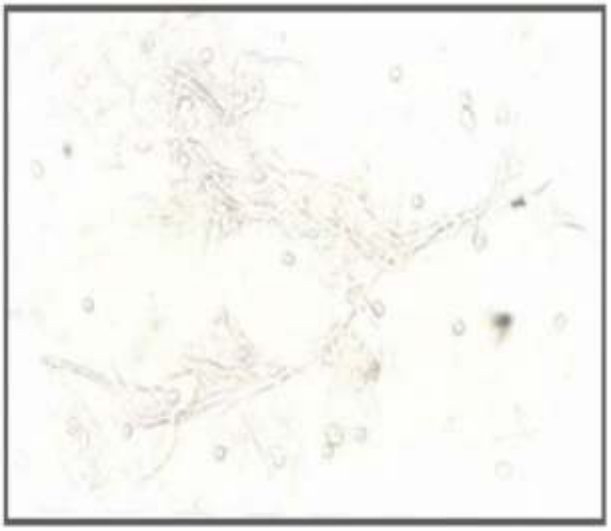


Figure 71A: Koh Preparation Of Vaginal Fluid Showing Budding Yeast And Mycelia

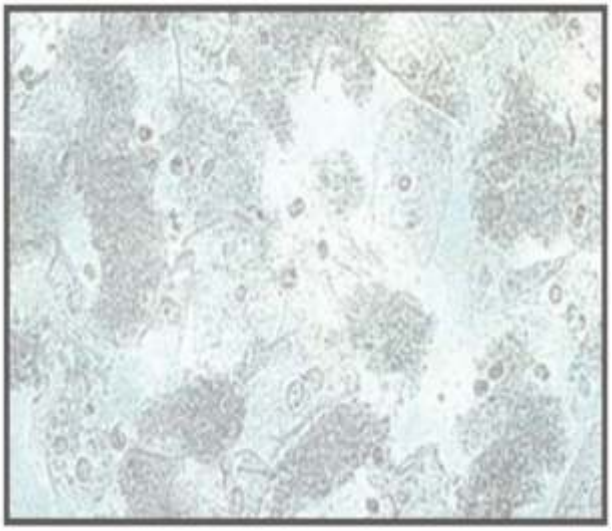


Figure 71B: "Clue Cells" In Vaginal Wet Mount (400X)

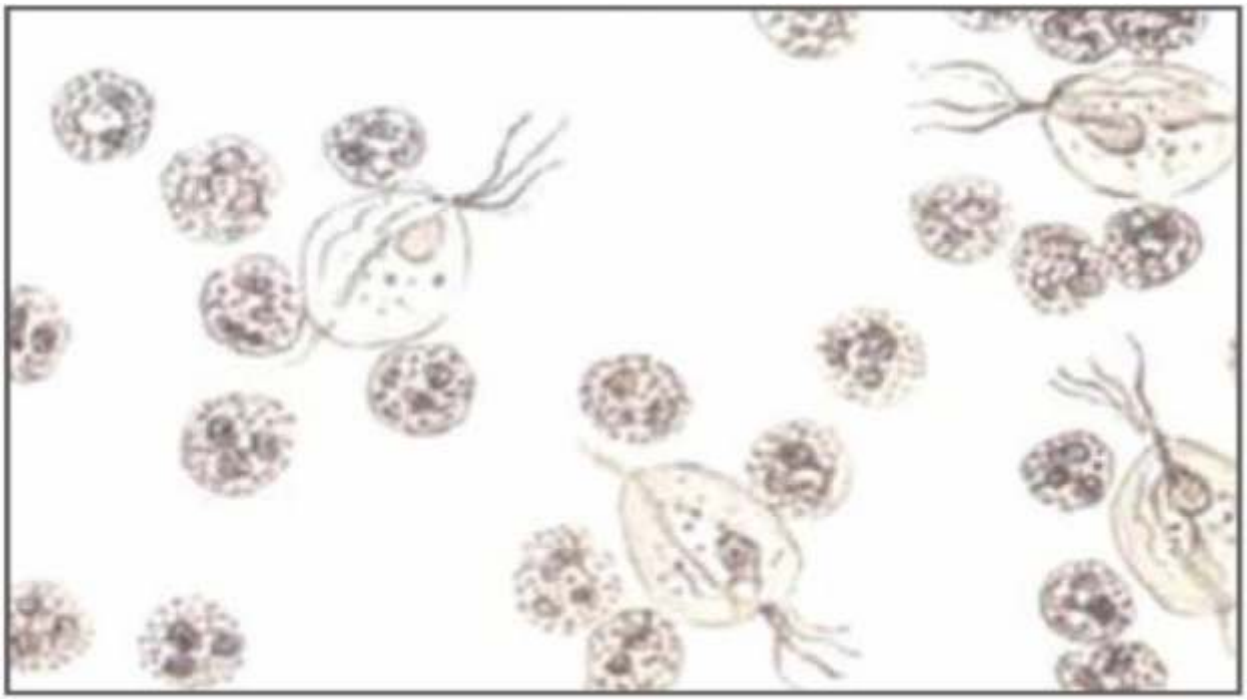


Figure 72: Trichomonas Vaginalis In A Wet Mount Of Vaginal Discharge (400X)
Source: Ministry of Health and Family Welfare (MoHFW). National Guidelines on Prevention, Management and Control of Reproductive Tract Infections Including Sexually Transmitted Infections. New Delhi: MoHFW; 2007.

Whiff Test

Women with BV often complain of a foul vaginal smell.

- This odor is due to the release of amines, produced by decarboxylation of the amino acids lysine and arginine by an aerobic bacteria. When potassium hydroxide is added to the vaginal fluid, these amines immediately become volatile, producing the typical fishy odor.
- Place a drop of vaginal fluid on a glass slide and add a drop of 10% potassium hydroxide. Hold the slide close to nose to detect the amine odor. After a positive reaction, upon standing, the specimen will quickly become odorless because the amines will be rapidly and completely volatilized.

Collect specimen	A Gram stain slide can be prepared at the same time as the wet mount by rolling the spatula/swab on a separate slide.
Prepare slide	1. Heat fix. 2. Stain with crystal violet (60 seconds) and rinse. 3. Stain with iodine (60seconds) and rinse. 4. Decolorize with acetone-ethanol for few seconds (until the liquid runs clear). 5. Stain with safranin (60 seconds) and rinse. 6. Gently blot dry and examine under oil immersion (1000X) and count each type of organism.
What to look for	· Lactobacilli only: Normal. · Mixed flora, mainly lactobacilli with a few short rods (coccobacilli): Considered normal. · Presence of clue cells; mixed flora, mainly Gardnerella and anaerobic bacteria with a few lactobacilli: Diagnose as BV. · Presence of clue cells, mixed flora of Gram-positive, Gram-negative, and Gram-variable rods; no lactobacilli: Diagnose as BV.
Important	Look for evidence of other vaginal or cervical infections as multiple infections arecommon.

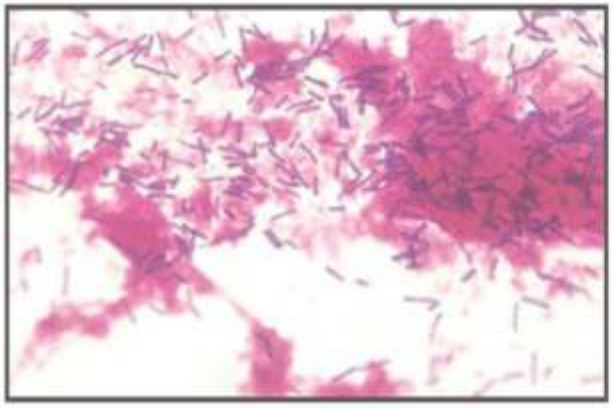


Figure 73A: Figure 72: Gram-Stained Vaginal Smear Showing A Normal Flora Of Lactobacilli (1000X)

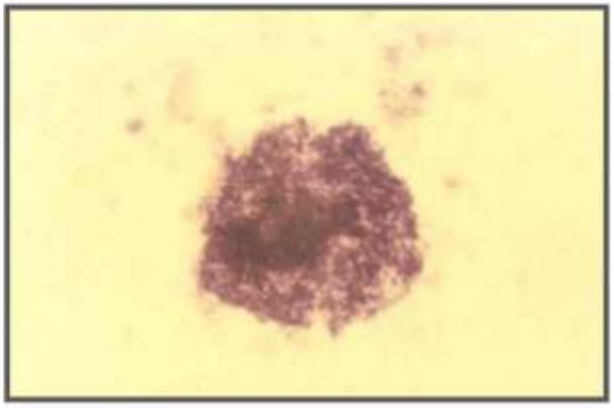


Figure 73B: Figure 73: Gram-Stained Vaginal Smear With Typical "Clue Cells" (1000X)

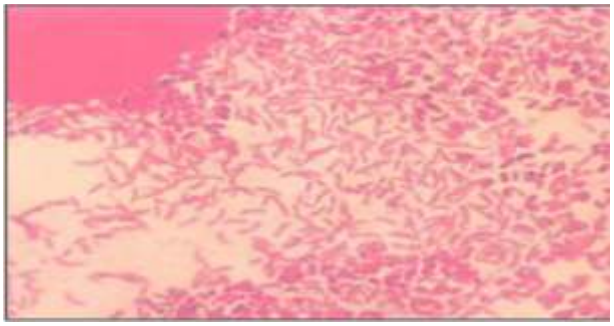


Figure 73C: Gram-Stained Vaginal Smear Showing Large Gram-Negative Rods

Figure 74: Figure 75: Gram Stain Smear: Gram Negative Diplococci Of Neisseria Gonorrhea

Source: Ministry of Health and Family Welfare (MoHFW). National Guidelines on Prevention, Management and Control of Reproductive Tract Infections Including Sexually Transmitted Infections. New Delhi: MoHFW; 2007.

Rapid Plasma Regain (RPR) test for Syphilis screening

1. Inform about the procedure of the test to be conducted. Seek consent.
2. Use a sterile needle and syringe. Draw 5ml of blood from a vein. Put in a plain test tube.
3. Let the test tube stand for 20 minutes to allow serum to separate (or centrifuge for 3–5 minutes at 2000–3000 rpm). In the separated sample, serum will be on top.
4. Use a sampling pipette to transfer the serum. Take care not to include any red blood cells from the lower part of the separated sample.
5. Hold the pipette vertically over a test card circle. Squeeze it to allow one drop (50µl) of serum to fall onto a circle. Spread the drop to fill the circle using a toothpick or other clean spreader.
6. Attach dispensing needle to a syringe. Shake antigen. *Draw up enough antigen for the number of tests done (one drop per test).
7. Holding the syringe vertically, allow exactly one drop of antigen to fall onto each test sample. Do not stir.
8. Rotate the test card smoothly on the palm of the hand for 8 minutes (or rotate on a mechanical rotator).

Figure 75: Figure 76: Test Serum Is Mixed With Antigen And The Card Is Placed On Appropriate Rotor

9. Interpreting results: After 8 minutes rotation, inspect the card in good light. Turn or tilt the card to see whether there is clumping (reactive result). Test cards include negative and positive control circles for comparison.
10. Non-reactive (no clumping or only slight roughness): Negative for syphilis
11. Reactive (highly visible clumping): Positive for syphilis
12. Weakly reactive (minimal clumping): Positive for syphilis

Figure 76: Reading Rpr Results For 10 Undiluted Sera Showing Reactive And Non -Reactive Samples. The Presence Of Small To Large, Flocculated Clumps Indicates Reactivity, Whereas No Clumping Or A Very Slight Roughness Indicates Non-Reactive.

Reference:

1. National Guidelines on RTI & STI. https://naco.gov.in/sites/default/files/National_Guidelines_on_PMC_of_RTI_Including_STI%201.pdf
2. https://naco.gov.in/sti-rti-services

Testing for Leprosy

Requirement: Gloves, Swabs and spirit, scalpel handle and new blade, dressing strip, sprit lamp, slides, slide box, safe disposal for box (sharp item disposal).

Skin smear examination

The skin smear examination is essentially three steps; first is the collection of the specimen, second is the staining of the slide and third is the microscopic examination of the material.

Reasonably accurate report is obtained in terms of positivity if proper technique is followed.

Step 1: collection of specimens

Note: Before taking each smear, wash hands and put on gloves.

Preparation of slide: Take a new, clean, unscratched microscope slide. Using a slide marker, write the patient identification (ID) number at the bottom of the slide. This number must be on the request form.

Collection of smears

1. Clean the skin at the smear sites with a cotton wad drenched in alcohol or spirit.
2. Allow it to dry.
3. Light the spirit burner.
4. Put a new blade on the scalpel handle. If you put the scalpel down, make sure the blade does not touch anything.
5. Pinch the skin firmly between your thumb and forefinger; maintain pressure to press out the blood.
6. Make an incision in the skin about 5 mm long and 2 mm deep. Keep on pinching to make sure the cut remains bloodless. If bleeding, wipe the blood with cotton wad.
7. Turn the scalpel 90 degrees and hold it at a right angle to the cut. Scrape inside the cut once or twice with the side of the scalpel, to collect tissue fluid and pulp.
8. There should be no blood in the specimen, as this may interfere with staining and reading. Stop pinching the skin and absorb any bleeding with a wad of cotton.
9. Seal the cut site with Tr. Benzoin
10. Spread the material scraped from the incision onto the slide, on the same side as the ID number.
11. Spread it evenly with the flat of the scalpel,making a circle 8 mm in diameter.

12. Rub the scalpel with a cotton wad drenched in alcohol.
13. Pass the blade through the flame of the spirit burner for 3 to 4 seconds.
14. Let it cool without touching anything.
15. Repeat the steps above for the second site. Spread this smear next to, but not touching, the first one.
16. Discard the scalpel blade safely. Thank the patient.

Fixation of smear on slide

1. Let the slide dry for 15 minutes at room temperature, but not in direct sunlight.
2. Fix the smears by passing the slide, with the smears upwards, slowly through the flame of a spirit burner, 3 times. Do not overheat.
3. The slide should not be too hot to touch. Put the slide in a slide box & send to the laboratory with the skin smear request form.

Step 2: Staining the smear (Ziel-Neilson Technique)

Stain the slide by following Ziehl-Neilson technique as described above.

Step 3: Microscopy: How to read smear

1. Put the slide under the microscope with smear upward and ID number o the left.
2. Focus the image using 10X eye piece.
3. Put a drop of immersion oil on the smear.
4. Switch to the 100X objective this will touch theimmersion oil.
5. Open the diaphragm completely and raise the condenser to its highest position.
6. Focus precisely with the fine adjustment screw. Look for the presence of acid-fast bacilli (appear red colored a fine rod against blue background) Table:

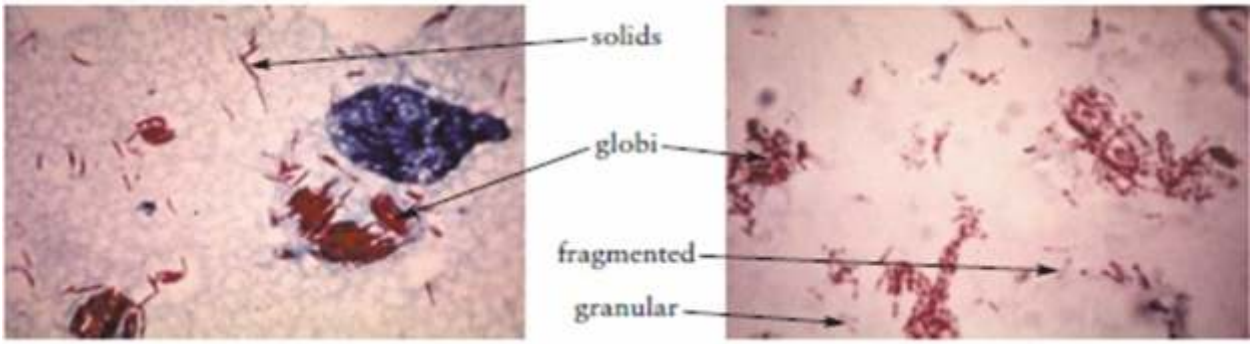


Figure 77: Figure 78: Acid Fast Bacilli
Source: https://images.app.goo.gl/u2en5gbQXrRTva4z5

Grading chart for ZN microscopy (100X oil immersion objective and 10X eye piece)

Zn Grading (NTEP)	Reporting Grading
>10 AFB/ filed after examination of 20 fields	Positive 3+
1-10 AFB/ filed after examination of 50 field	Positive 2+
10-99 AFB/100 field	Positive, 1+
1-9 AFB/100 field	Positive, Scanty
No AFB per 100 fields	Negative

Smear Preparation and staining of other samples

- **Swabs-** If two swabs are provided one is cultured directly while the other is used for making smear. Roll the swab lightly on the clean glass slide. If only one swabs is taken, first it should be cultured and then used for smear examination.
- **Pus and discharges –** spread over a clean slide. The smear should not be very thick.
- **Sputum samples** -Same as pus but if mucous is present it should taken as a representative sample.
- **Clear fluids and CSF** – Take a loopfull on a clean glass slide and let it air dry air dry as such without spreading.
- **Cultures** - Take a loopful of broth culture and let it air dry OR take a loopful of sterile normal saline on a slide. Touch the colony to be examined with the loop and gently form a suspension on the slide with the N. saline while spreading it out (1x2 cm)

Albert Stain	
Albert Solution I	
Toluidine blue	0.15 gm
Malachite green	0.2 gm
Glacial acetic acid	1 ml
Alcohol (95% ethanol)	2 ml
D/W	100 ml
Dissolve toluidine blue and malachite green in alcohol and then add water, acetic acid. Allow tostand for one day and then filter it.	

Albert solution II	
Iodine	2 gm
Potassium Iodide (KI)	3 gm
D/W	100 ml
Dissolve KI in water and then add Iodine. Dissolve iodine in potassium iodide solution	

Albert Staining for Diphtheria

The smear is made similarly, Impressions are made on 2 slides - 1 for Gram's stain and another for Albert's stain. After drying and heat fixing

Add Albert's I stain x 5-7 minutes and wash with tap water

Add Albert's II stain x 1-2 minutes and wash with tap water.
Gently blot dry it and examine

Wet Preparation of Urine

1. Take a drop on the clean glass slide (uncentrifuged) and gently put a clean cover slip over it so that no air bubble is trapped in between.
2. Examine under the 10x lens first and then 40x with the condenser lowermost and light diminished.
3. One pus cell/hpf and one bacteria/oil if is significant.

Hanging Drop of Stool

It is used for direct stool exam of suspected cholera case and motility exam of bacteria from broth cultures.

1. Make a thin plasticine ring in the centre of a clean slide.
2. Place a cover slip on a small piece of paper on the table.
3. Place a loopful of suspected stool sample in the middle of the cover slip.
4. Invert the slide with the plasticine ring over the cover slip preparation and gently press to allow the coverslip to stick to the ring.
5. Gently turn the slide upside down so that the coverslip is upwards.

6. Examine under 10 x and focus on the edge of the fluid. Then shift to 40x and examine.

Blood Culture

- Blood is sent in BHI broth to the laboratory.
- On Receipt- On special request, a smear from the culture can be made if growth is suspected on receipt of the sample.
- Kept in the incubator 37C.
- 24 hours - S/c done on Blood Agar and MacConkey Agar. If negative
- 48 hours - S/c done similarly.
- 10 days - Final S/c on BA and MA in case of positive cultures proceed for identification and antibiotic sensitivity report.
- Cultures observed- 3 weeks in Infective Endocarditis patients where more than one sample is received.

Reporting Blood culture Results

- Wherever a growth of suspected pathogen is detected, it should be communicated. In the case of a suspected contaminated sample e.g., ASB (aerobic spore bearing) or more than 3 types of bacteria, diphtheroid, or Coag Neg Staph a repeat sample should be requested.
- If Salmonella is suspected slide agglutination done and reported.
- Final Report
- 48 hours if no growth is suspected. In case of positive cultures > The final report with identification and sensitivity is dispatched.
- Additional Report
- The report after 10 days and 3 weeks is sent only if positive for suspected pathogenic bacteria.

Stool Culture:

Direct Hanging drop if V. cholerae is suspected. If positive, immediately send a preliminary report

Isolation

- Direct inoculation on MA and DCA, BA+TCBS if V. cholera is suspected
- BA inoculated when membranous colitis is suspected
- Inoculate into Selenite `F' enrichment broth.
- Alkaline peptone water if V. cholera is suspected (re-examine after 3-4 hrs of incubation)

24 Hrs

- Suspected Salmonella/Shigella colonies are identified and reported
- Pure growth of any other Gram Neg. Bacteria in Children less than 3 years is identified and reported.
- Pure growth of staphylococci is reported
- Vibrio cholerae is reported. If present

48 Hrs

- If none of the above is positive - Report as "No Salmonella/Shigella/ Vibrio grown.
- A comment on normal flora can be made, if relevant.

DCA- deoxycholate citrate agar, TCBS- Thiosulfate– citrate–bile salts–sucrose agar

CSF Culture

On receiving- Centrifuge at 1500 rpm		
a) Deposit: Gram smear and Culture BA, CA and BHI broth	b) Supernatants: Shift in a sterile vial and can be usedfor Ag detection	c) Add the rest to the depositand incubate at 37C
↓		
Keep the sample + Plates + Broth at 37C. CA in incubated in candle jar.		
Smear report as - Pus cells and Bacteria present (Specify morphology and gram stain)	If smear suggestive of menin - gitis - direct sensitivity may be put up	Convey the preliminary reporton telephone
↓		
24 hours - Observe cultures		
If positive - Identify, do the antibiotic sensitivity and sendthe report.	If negative: Reincubate , look for any turbidity in broth or CSFand if present get a S/c done	Subculture if direct smear is positive
↓		
After 48 hrs- Report final as Negative In case of Coagulase negative staphylococcus, Diphtheriods or othersuspected contaminants ask for repeat sample Diphtheroids should be differentiated from Listeria species		

Nasal Swab/ Throat Swab Culture

Throat swab: Direct smear is only made if throat swab from suspected. Diphtheria case is sent Albert's stain is done and preliminary report sent.

Inoculate BA
↓
If Diptheria is suspected Inoculate Loeffler's serum slope, 1/2 BA and 1/2 Potassium tellurite medium. Subculture from Loeffler's slope into the rest of 1/2 of KT and BA. Preferably within 4-6 hours of inoculation.
↓
Process B haem streptococci, if grown. If isolation and grouping needs further 24 hours, then a preliminary report should be despatched.
↓
In patients where chances of aspiration pneumonia are high process pure cultures of Strep. viridans,Gram neg. bacteria, or Streptococcus pneumoniae. In immunocompromised patients. Also report of any alteration in normal flora.
↓
If only normal flora: Report as Non-B haemolytic streptococci grown Normal. No other common respiratory. pathogen grown.
↓
If no growth is seen -Inappropriate sample? dry swab, Ask for repeat sample.

Pus, Exudates and Wound Swab, Catheters/ Drainage Tubes, Tissue

If 2 swabs are received (A)	If only 1 swab is received (B)	If pus discharge/ aspirate/any other aspirate/ any other infectedfluid is sent (C)	If catheter or tube is sent (D)	If tissue received (E)
• One is cultured on Blood Agar (BA) and MacConkey Agar (MA) • The swab is inoculated in TGB medium • The other swab is used for preparing the direct smear	• It should be first cultured and then processed for smear	• 1 loopful is used to inoculate BA and MA and one loopful is put in TGB • 1 loopful is used for direct smear	• Roll the catheter all over with the help of a sterile forceps on the BAand then MA • The tube is then put into the TGB. • > 15 colonies of bacteria growing on the plate are significant	• The tissue should be minced in a sterile tissue grinder. Then process as in C

Nasal swab/ Vaginal swab/ endo cervical swab culture

Nasal Swab	High vaginal Swab/Endo-Cervical Swab
No smear is indicated: Culture on BA and MAReport 1) If Staphylococcus aureus grows in culture 2) Otherwise report as normal flora	1. Smear may be made if indicated and on special request. 2. Culture: Inoculate on BA and MA. Inoculate TGB. 3. If 24 hrs - sterile then S/c from TGB = If still sterile after 48 hours report. 4. Process a) Pure GNB (1 types or 2 types)B haemolytic group B streptococci 5. Mixed flora should be reported as (3 or >3 types) sample contaminated.

Culture Reporting

Direct Smear If positive and suggestive of presence of bacteria

24 Hrs culture- Growth	• Identify and do AST if 1 or 2 types of org grown
Possible contamination	• 3 or more types of organism grown- difficult to put AST • In case of diphtheroids - report as possible contaminants
Culture sterile at 24 hrs	• Examine Thio glycolate broth (TGB) • If Turbid - Subculture into BA and MA (may not give a true picturein case of catheters where quantitative assessment is done) • Meanwhile also re-incubate the original plates

Bunsen Burner

Bunsen burner is used to create sterile environment while media preparation/ pouring, slide staining, reagent preparation.

- Bunsen burners are free-flame devices utilized to carry out small weldings and heatings
- In order to avoid any risks, the Bunsen burner has to be connected properly to the natural gas distribution

network or to the liquid gas bottles; connection has to be carried out by means of type-tested pipelines according to the type of gas being used and duly fixed in place with suitable hose clamps.

MAINTENANCE: To preserve the highest and long-lasting working efficiency of your bunsen burner, it is necessary to keep clean the outlet hole of the bunsen pipe.

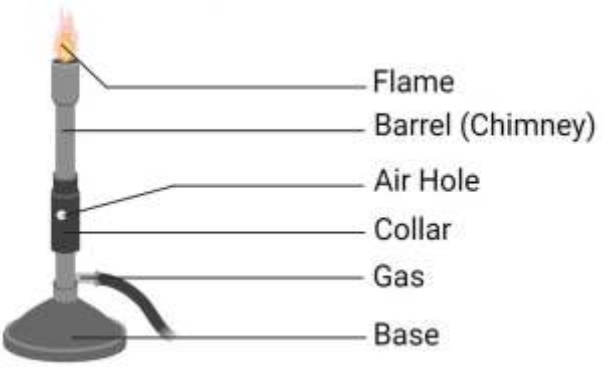


Figure 79: Bunsen Burner
Source https://images.app.goo.gl/7xdXAm- 7muC2pZUPM8

Cautions of handling Bunsen Burner

- The risk of fire is flammable reagent exposed.
- Always keep away the alcohol from the flame of Bunsen burner
- Traces of alcohol from hand must be removed while handling material near the flam of Bunsen burner.
- Do not use Bunsen burner in Biosafety cabinet. Flam may lead to damage of HEPA filter

Incubator

Incubators are necessary to maintain constant temperature within the range for optimum growth.

- Ensure that the incubator is connected to the mains.
- Keep door(s) closed.
  - o Prevents heat loss.
  - o Keeps temperature stable.
- Do not place containers of media too close together.
  - o Space is needed for adequate air and temperature circulation.
- Label racks with week of incubation
  - o Enables efficient checking for growth ever yweek for the 6–8-week incubation period
- Record temperature twice daily. i.e. in the morning and in the evening. The temperature should not differ ± 2° C from the set temperature.
- Do not use an incubator in the presence of flammable or combustible materials as components inside of the incubator could act as ignition sources during operation.

Figure 80: Incubator
Source: https://images.app.goo.gl/QYhTRh6YMc3XV82X8

Maintenance of incubator

- Record temperatures daily
  - o Use a separate thermometer stored in a sealed glycerol container.
  - o Calibrate thermometer every year
- Clean with disinfectant immediately after any infectious spills
- Clean thoroughly with disinfectant on a monthly basis

Calibration of Incubators

- Temperature in the incubator has to be calibrated for the critical range. We need to set the incubator at the correct temperature.

- Turn on the incubator and set the temperature control knob; leave a thermometer in a glycerol container inside away from the heating element, close the door and let the temperature stabilize for at least one hour before beginning the procedure.
- Check the reading on the thermometer. Adjust the temperature control knob, if the temperature is not within range. Close the door and recheck in 30 minutes.
- Repeat procedure until two consecutive readings are in the range.
- Record all readings in the incubator logbook.
- Mark the temperature control knob at the position where the temperature is in range.
- Incubators need to be calibrated when installed.

Thermal Cycler

The Thermal Cycler has several molecular biology applications, including in Polymerase Chain Reactions (PCR)

- To avoid electric shock, make sure the machine is plugged into a grounded electrical outlet.
- Ensure adequate ventilation by placing a Thermal Cycler at least 30 cm away from adjacent instruments or walls.
- Place Thermal Cycler on a firm and flat surface capable of withstanding the weight of the machine.
- Machine should be installed in room with ambient temperature 15-30°C and relative humidity 20-80% and location free of excessive dust.
- Do not use the machine in a potentially explosive environment or with potentially explosive chemicals.
- Repairs should be carried out by authorized service personnel only.

Figure 81: Thermal Cycler
Source: https://images.app.goo.gl/XAZMxA9euBMqXkKR6

Maintenance- Thermal Cycler

- Periodically use soft lint-free cloth and distilled water to wipe unit clean of dust.
- A heated lid may be cleaned by sliding the lid back on the tracks until the front pins align with slots, holding the rolleiflex cable clear.
- Lift lid from the front and flip it up. Use mild detergents to clean lid. A Kim wipe (tissue paper) dipped in 70% ethanol can be used to remove residue from sealing tape.

- The frame of unit may be cleaned with slightly moist cloth or ethanol. Avoid aggressive cleaning agents.
- Servicing
- The thermal cycler must only be opened by an approved expert.
- In case of damage or failure to work, an Equipment Failure Report must be completed and the authorized service representatives must be called out.
- An Equipment Failure Notice must be placed on the thermal cycler indicating that work is prohibited.

Table 11: Thermal Cycler Error Messages

Message	Cause	Action
HEATE R ERROR!	· Lid temperature sensor problem. · Lid temperature exceeds 132 °C. · Lid temperature can't reach the set temperature.	Reboot the unit
BLOCK ERROR!	· Block temperature sensor or heating element problems. · The unit can't reach the set temperature, because the room temperature is too high. · The block temperature exceeds 108°C	Cool down the room temperature. Reboot the unit
ROOM ERROR!	· Room temperature sensor problem	Reboot the unit
Room > 30^{0}C	· The room temperature exceeds 30°C. · Two running units are too close to each other	Cool down the room temperature. Reboot the unit. Remove any objects blocking the vents. Keep two running units at least 30cm from each other

Table 12: Troubleshooting- Thermal Cycler

Problem	Cause	Action
The display is off even when the poweris switched on.	1. Power is not reaching the system. 2. The power cord is not plugged into the socket properly. 3. The power fuse is blown out. 4. Block fuse is blown out.	5. Check power source voltage. 6. Reconnect the power cord. 7. Replace the power fuse. 8. Return the unit for service
Can't reach 4°C.	1. Operating temp environment may be unsuitable. 2. Electronic cooling element may be damaged or aged	3. Operate the unit in a environment temperature between 15 to 30 °C. 4. Return the unit for service.
Cycle time is too long	1. Operating environment temperature may be unsuitable. 2. Electronic cooling elements may be damaged or aged. 3. Faulty temp sensor.	4. Operate the system in a temperature environment between 15 to 30 °C. 5. Return the unit for service. 6. Return the unit for service.

Lid heater does not work.	1. The lid heater is set off. 2. Lid sensor problem	Check Lid Temperature setting in System Mode. Return the unit for service.
No key entry sound.	1. Sound may currently be set off. 2. Faulty keys or keypad wiring.	Check Beeper setting in System Mode. Return the unit for service.
The display goes off.	1. Faulty backlight. 2. Faulty LCD panel	3. Check the LCD contrast. 4. Return the unit for service
Display is too dark or light	Backlight brightness is not adjusted properly.	Adjust Display Brightness Potentiometer.
Lid will not close.	Foreign matter, between lid heater and sample block. Faulty lid lock mechanism.	Remove the foreign object or matter.
Error message appears.	Refer to list of error messages below.	Check the nature of the error and takethe suggested actions

Micropipette

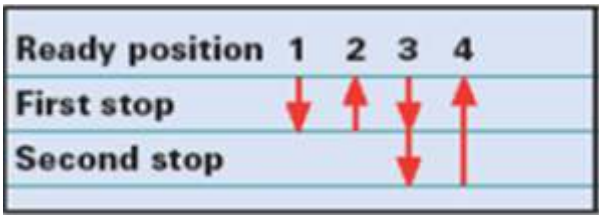
A variety of pipettes are used in laboratory procedures for accurate, precise sampling and dispensing of liquid volumes. They operate on the air displacement principle.

- Fixed volume and variable volume pipettes can be subdivided into two types: A and B.
- Pipette A: Also named air displacement pipettes due to the fact that there is a volume of air between the head of the piston and the liquid in the cylinder. These are meant for general use with aqueous solutions.
- Pipette B: Also called positive displacement pipettes or direct displacement pipettes as the piston is in direct contact with the liquid. These are used for high viscosity and volatile liquids.

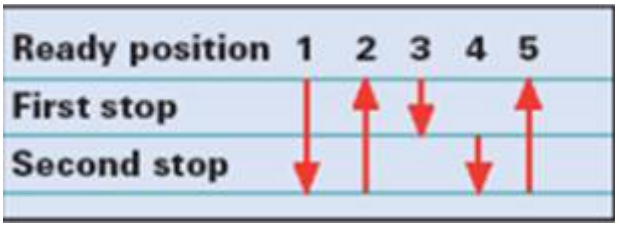
Figure 82 : Micropipette
Source: https://images.app.goo.gl/9UdSNcdBq9TrMuzi9

Aspirating and dispensing the liquids

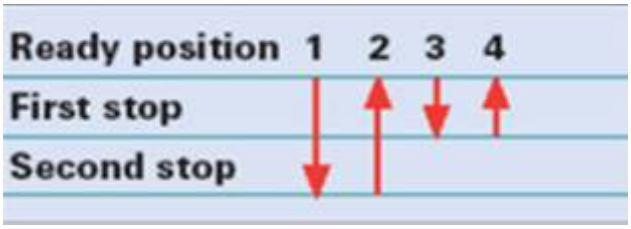
- Hold the pipette vertically when aspirating liquid and place the tip only a few millimeters into the liquid. Push the thumb button to first stop.
- For dispensing the entire volume taken in the tip, press the button to the second stop. The pipette's tip must form an angle ranging between 30 and 45° with the tube at 8 to 10 mm above the surface of liquid



Forward Pipetting



Reverse Pipetting



Repetitive Pipetting

- Always control the push button movements with the thumb to ensure consistency.

Maintenance and Validation of pipettes Maintenance

- Regular disinfection before and after each procedure
- ALWAYS use clean filter tips
- Validation – every 3 to 4 months
- Clean with 70% alcohol

Validation

- Verify that the correct volume is being pipetted.
- Validate accuracy and precision.
- Electronic balance needs to be "centered" and validated with certified weights before validation of pipettes can be done.

Table 13: Recommendations for Pipetting different Solutions / Compounds

Solution/ Compounds	Examples	Pipette	Tip	Technique	Comments
Aqueous Solution	Buffers, diluted salt solution	Air Displacement	Standard	Forward	
Viscous Solution	Protein, nucleic acid, glycerol, Tween 20/ 40/ 60/80	Air Displacement Pos Displacement	Standard wide orifice Positive Displacement	Reverse	Pipette slowly to avoid bubble formation
Volatile compounds	Methanol, hexane	Air Displacement Pos Displacement	Filter, Positive Displacement	Forward	Pipette rapidly to avoid evaporation. Carbon filter tips prevent vapors from going into thepipette
Nucleotide Solution	Genomic DNA, PCR products	Air Displacement Pos Displacement	Filter, or wide orifice Positive Displacement	Forward	For genomic DNA wide orifice to be used to avoid mechanical shearing
Acid/Alkali	H_2SO_4, HCL, NaOH	Air Displacement	Filter	Forward	

pH Meter

- The pH meter is used for determining the concentration of hydrogen ions [H+] in a solution.
- This equipment, provided it is carefully used and calibrated, measures the acidity of an aqueous solution.

pH meter calibration

One point calibration

- · This is carried out for normal working conditions and for normal use.
- · It uses one known pH reference solution.

Two-point calibration

- This is done prior to performing very precise measurements.
- It uses two known pH reference solutions.
- It is also done if the instrument is used sporadically, and its maintenance is not carried out frequently.
- Calibration frequency: Daily

Figure 83: Ph Meter
Source: Maintenance Manual for laboratory equipment, WHO, 2nd Edition

Cleaning of electrode

- **General cleaning**: Soak the pH electrode in a 0.1 M HCl solution or 0.1 M HNO3, for 20 minutes. Rinse with water.
- **Removal of deposits and bacteria**: Soak the pH electrode in a diluted domestic bleach solution (e.g. 1%), for 10 minutes. Rinse abundantly with water
- **Cleaning oil and grease**: Rinse the pH electrode with a mild detergent or with methyl alcohol. Rinse with water
- **Cleaning of protein deposits**: Soak the pH electrode in 1% pepsin and 0.1 M HCl for 5 minutes. Rinse with water.

Table 14: pH Meter Troubleshooting

Problem	Probable Cause	Solution
The pH meter shows unstable readings.	There are air bubbles in the electrode.	Soak the electrode to eliminate the bubbles.
	The electrode is dirty.	Clean the electrode and recalibrate.
	The electrode is not immersed.	Verify that the sample covers the tipof the electrode perfectly.
	The electrode is broken.	Replace the electrode.
The electrode's response is slow.	The electrode is dirty or greasy.	Clean the electrode and recalibrate.
The screen shows an error message.	Incorrect operating mode selected.	Verify the operation mode selected. Select a valid operation.
The screen shows a calibration or error message.	There is a calibration error.	Recalibrate the pH meter.
	The calibration of the buffer value is erroneous.	Verify the buffer values used.
	The electrode is dirty.	Clean and calibrate the electrode.
The pH meter is on, but thereis no signal on the screen. (Battery operated pH meters only)	The batteries are badly installed.	Verify the polarity of the batteries.
	The batteries are worn out.	Replace the batteries.
The battery indicator is flashing. (Battery operated pH meters only)	The batteries are worn out.	Replace the batteries.

Balances

- A balance is used to accurately measure quantities for reagent and media preparation.
- A digital balance where there is one significant figure after the decimal point is suitable for reagent preparation.
- Follow the manufacturer's directions for the use and maintenance of the balance.
- Check the external drift of the balance each day of use with an external weight. Document results in the usage log.
- Read and understand the manufacturer's instructions.
- Calibrate each time of use with a set of known weights.
- Brush off dry spills and use a damp cloth to clean liquid spills.
- Document all calibrations and cleanings on the log.

Figure 84: Electronic Balance
Source: Maintenance Manual for laboratory equipment, WHO, 2nd Edition

Table 14: Balance troubleshooting

Problem	Probable Cause	Solution
The balance does not turnon.	The interconnection cable is disconnected or maladjusted on the balance.	Check the connection. Adjust the cable connector if this is thecase.
	Electrical outlet has no power.	Check electrical feed.
The weight reading is incorrect.	The balance was not adjusted to zero before the reading.	Place the balance on zero; repeat the measurement.
	The balance is incorrectly calibrated.	Calibrate according to the procedure recommended by the manufacturer.
The balance does not show the desired unitsof measurement on the screen.	The units are incorrectly selected.	Check the procedure defined by the manufacturer, select the required measurement unit.
	The unit required not available or not activated.	Activate the measurement unit according to the procedure defined by the manufacturer
The balance's reader is unstable.	There is vibration on the surface ofthe table/counter.	Place the balance on a stable surface.
	The front door of the balance is open.	Close the front door to measure.

Thermometer

- Verify all thermometers in the laboratory are accurately reflecting the temperature.
- A calibrating thermometer is used to check the readings on the thermometers. The temperature reading on the calibrating thermometer and the test thermometer must agree within +/- 1 degree.
- Document the calibrating thermometer and test thermometer results on the thermometer calibration log. Calibrating thermometers are certified by the manufacturer to accurately read the temperature and they need to be sent back to the manufacturer for recertification very year.
- Before relying on a thermometer to reflect the temperature, verify the readings by placing the thermometer and the calibrating thermometer in the same equipment and close together for about one hour.
- Record the temperature on both thermometers on the log sheet. The temperature on the test thermometer should be within 1°C of calibrating thermometer. Repeat if difference is greater than 1°C. Discard thermometer if repeat test failed.
- Document all results.
- Thermometers that are in use are calibrated every 6 months and documented.
- Spirit filled thermometers are preferred over mercury filled thermometers for safety reasons.
- Mercury is an environmental and health hazard that requires special handling and disposal for broken mercury thermometers.

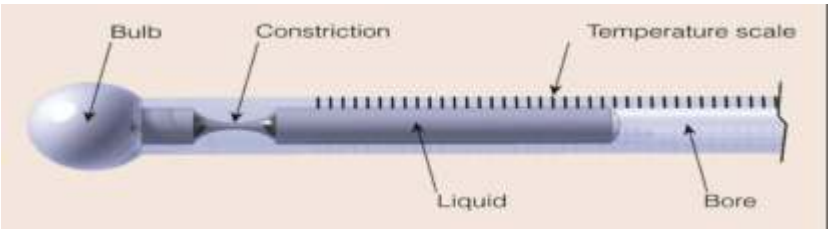


Figure 85: Mercury Thermometer
Source: https://images.app.goo.gl/1jss6hd95LJfNGQn8

Figure 86: Digital Thermometer
Source: https://images.app.goo.gl/BgrrycmPRMbHQpKNA

ELISA

Purpose

- To determine the optimal concentration of a relevant capture antibody or soluble antigen.
- To determine the optimal concentration of a lot of specific enzyme linked secondary antibody.
- To determine the suitability for use of a lot of substrate buffer as measured against a lot of known performance.
- To determine the suitability for use of a lot of PNPP substrate as measured against a lot of known performance.

Equipment required for ELISA testing.

1. Microplate reader/ ELISA reader
2. Microplate washer
3. Multi-channel pipettes
4. Incubators to incubate plates.

ELISA Procedures

1. The plate wells are coated with antibodies or antigens.
2. Samples, controls and standards are added to the wells and incubated at temperatures ranging between room temperature and 37°C for a determined period of time, according to the test's characteristics. During the incubation, the sample's antigen binds to the antibody coated to the plate; or the antibody in the sample binds to the antigen coated on the plate, according to their presence and quantity in the sample analyzed.
3. After incubation, the unbound antigen or antibodies are washed and removed from the plate by the microplate washer using an appropriate washing buffer.
4. Next, a secondary antibody, called the conjugate, is added. This harbors an enzyme which will react with a substrate to produce a change of color at a later step.
5. Then begins a second period of incubation during which this conjugate will bind to the antigen-antibody complex in the wells.
6. After the incubation, a new washing cycle is done to remove unbound conjugate from the wells.
7. A substrate is added. The enzyme reacts with the substrate and causes the solution to change in color. This will indicate how much antigen- antibody complex is present at the end of the test.
8. Once the incubation time is completed, a reagent is added to stop the enzyme-substrate reaction and to prevent further changes in color. This reagent is generally a diluted acid.
9. Finally, the plate is read by the microplate. The resulting values are used to determine the specific amounts or the presence of antigens or antibodies in the sample.

Types of ELISA

Direct ELISA: In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA. It is used in assessing antibody affinity and specificity. Investigating blocking/inhibitory interactions.

Advantage: Fast and Sample.

Disadvantage:

- Less specific since using 1 antibody.
- Potential for high background if all proteins from a sample are immobilized in well.

Indirect ELISA

An indirect ELISA is similar to a direct ELISA in that an antigen is immobilized on a plate, but it includes an additional amplification detection step. First, an unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the host species of the primary antibody is then added. The substrate then produces a signal proportional to the amount of antigen bound in the well. Used in measuring endogenous antibodies.

Advantage: Amplification using a secondary antibody

Disadvantage: Potential for cross-reactivity caused by secondary antibody

Sandwich ELISA

Sandwich ELISAs are the most common type of ELISA. Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated- detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.

It is used in determining analyte concentration in a biological sample.

Advantages:

· Highest specificity and sensitivity

· Compatible with complex sample matrices

Disadvantages:

· Longer protocol

· Challenging to develop

Competitive ELISA

Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. Similar to a sandwich ELISA, a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present

in the sample. The more antigen present in the sample, the less conjugated antigen will bind to the capture antibody. Substrate is added and the signal produced is inversely proportional to the amount of protein present in the sample. It is used in determining concentrations of small molecules and hormones.

Advantages: Ability to quantitate small molecules

Disadvantages:

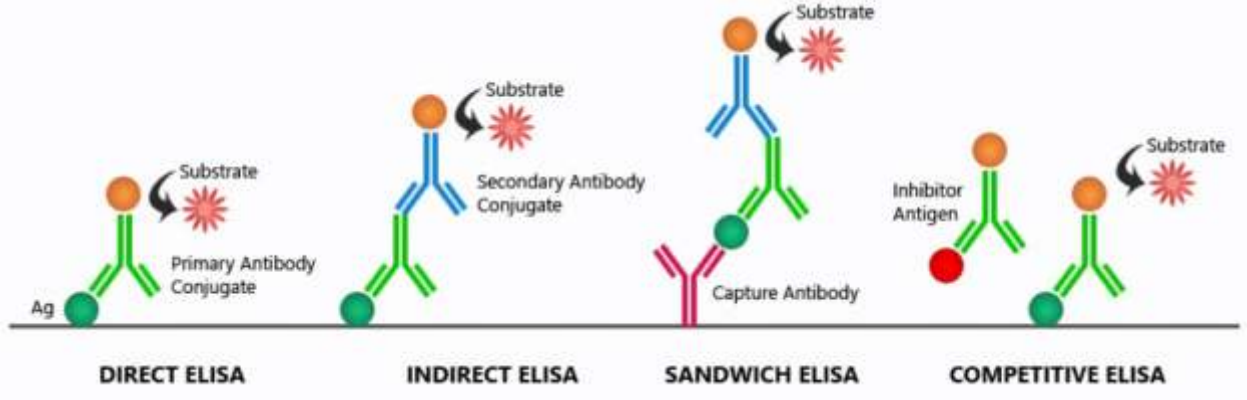
· Less specific since you are only using 1 antibody.

· Requires a conjugated antigen.

Microplate reader/ ELISA reader

- Working is based based on spectrophotometer's principle.
- Technique used to determine the presence of antibodies or specific antigens in samples.
- The technique is based on the detection of an antigen or antibodies captured on a solid surface using direct or secondary, labelled antibodies, producing a reaction whose product can be read by the spectrophotometer.
- Microplate reader has filters or diffraction gratings that limit the wavelength range to that used in ELISA, generally between 400 to 750 nm (nanometres)
- The optical system exploited by many manufacturers uses optic fibers to supply light to the microplate wells containing the samples.

- The light beam, passing through the sample, has a diameter ranging between 1 to 3 mm.
- A detection system detects the light coming from the sample, amplifies the signal and determines the sample's absorbance. A reading system converts it into data allowing the test result interpretation. Some microplate readers use double beam light systems.



Source: https://images.app.goo.gl/r139s2onJLki3wdz7

Microplate Washer

- The microplate washer has been designed to supply cleaning buffers required during the washing process. for the ELISA technique in a controlled manner.
- In the same fashion, the equipment removes from each well substances in excess from the reaction.
- Depending on the test performed, the washer can intervene from one to four times, supplying the washing buffer, agitating and removing the unbound reagents until the programmed times and cycles are completed.
- The washer has two reservoirs; one for the washing buffer, the other for the waste generated.

Figure 87: Elisa Reader
Source: Maintenance Manual for laboratory equipment, WHO, 2nd Edition

CHAPTER - 17

EQUIPMENTS AND TESTS IN LABORATORY

HEMATOLOGY ANALYZER

Purpose: To describe the procedure for the operation and maintenance of the Hematology Auto Analyzer

Scope: This SOP describes how to use and maintain a Hematology Auto Analyzer. This performs automated blood counts and requires no manual operations for aspirating blood, dilutions, measuring, calculations, printouts and computer transfer used in the Biochemistry laboratory.

Responsibility: It is the responsibility of Lab – In- charge of the lab to train Lab Assistant and students on this procedure and to ensure adherence to this procedure. It is the responsibility of the students/ technicians to follow the SOP as described and to inform the Lab In-Charge about any deviations or problems that may occur while performing the procedure.

Standard Operating Procedure for Hematology Auto Analyzer

Specimen requirements:

About 2-3 ml of venous blood collected into EDTA tubes. Specimens should be transported at room temperature 18 - 26^{0}C and can be stored in the refrigerator of 2 - 8^{0}C for up to 6 hours. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis.

Procedure:

1. Check the operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. The instrument performs an initialization phase for the internal electronics. Please wait.
4. Once the initialization phase is complete, the analyzer will automatically run a startup cycle.
5. If it does not automatically run a startup cycle after the initialization phase is completed, press the "Startup" button in the "Status" area to initiate a startup cycle.
6. Then, the instrument will perform a blank cycle for a reference blank count (an analysis cycle based on reagents without any blood sample).
7. Check and verify that the reference blank counts do not exceed the following parameter limits: WBC < 0.3, RBC < 0.02, HGB < 0.3, PLT < 10 then: Press "OK" button to validate blank results.
8. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
9. Entering patient ID, sample ID, Patient name, etc
10. Follow the indications displayed in the "Sample analysis" dialog box to run the analysis.
    a) Mix the sample gently and thoroughly.
    b) Remove the cap from the sample tube.
    c) Place the sample beneath the sampling needle.

d) Raise up the tube so that the sampling needle lowers into the blood and press the manual sample bar.

e) The analysis cycle will begin.

11. When the analysis is completed, the "Sample analysis" dialog box is closed, and results are displayed in the "Result display" menu for print out.

Precautions to be followed

Criteria for rejection hematology specimens

- o When the identification is missing /inadequate.
- o Insufficient quantity.
- o Inappropriate container
- o In appropriate transport/storage.
- o Unknown duration of delay.
- o Clotted sample.

Equipment

Record to be Maintained.

- Laboratory Manual containing the experiments that can be performed with the equipment.
- Maintenance Record

About Hematology Analyzer

The Analyzer

The obvious part is the physical hardware, including pumps, valves, counting mechanisms, electronics, and software, which generate measurements.

Hematology analyzers use various methods like impedance, optical light scatter, laser light scatter, and fluorescent flow cytometry to obtain results. Each method has strengths and limitations for analyzing fresh whole blood and stabilized materials for internal quality control.

The Reagents

These dilute the specimen, preserve the cells, convert hemoglobin into a measurable form and allow the analyzer to do its job. Different cellular analysis technologies require development and use of specific reagents. These reagents may react differently with fresh whole blood and fixed control bloods.

The Calibrator

This material acts much like real blood. In fact, it is manufactured using real blood in a very long and complicated process. It is used to set the system to known values. Normally calibration or calibration verification is done infrequently. Assayed whole blood may also be used to calibrate an analyzer.

Calibration

Before starting, the system needs to be calibrated. This is to make sure that the calibration factors which ensure correct results are set to the proper values. As a part of the setup procedure of your new system, the Service Engineer or Application Specialist may have completed the calibration for you. If not, or if something has changed with the system, you will have to perform a calibration. To calibrate your system, follow all directions in the Operations Manual, as well as the instructions included with the calibration material.

When to calibrate the CBC analyzer?

Calibration and/or calibration verification is recommended:

- When a change in the reagent type from the same vendor occurs or a change to a different vendor occurs
- When verification is indicated by trends in quality control data
- After major maintenance and service procedures
- At least every six months or as required by the regulatory agencies that govern the laboratory.

The Controls

Commercial controls are assayed to obtain target means (values and ranges) for either a single or multiple hematology systems by using instruments that have been calibrated to reference methods.

The quality control material you use also mimics, as closely as possible, the characteristics of real blood.

Running Controls

After calibration, it is required to run the controls. This will confirm that the calibration was performed correctly, and the system is operating properly.

Frequency of running controls in routine practices

How often laboratory personnel run the controls will depend on laboratory's operating schedule and policies, as well as applicable regulatory requirements. **It is common, and good laboratory practice, to run the three levels of control at least once on each work shift**. However, your schedule may vary, and some labs run controls more often, others less.

Types of Controls

Two types of quality control materials are used: commercial controls and retained patient specimens (secondary controls). Many laboratories use both types of control materials.

Commercial Controls

Commercial controls are made from pooled whole blood that has been preserved to delay deterioration. The manufacturer provides an expiration date for each lot of control material, which applies only to unopened bottles. Opened containers have their own open stability dates.

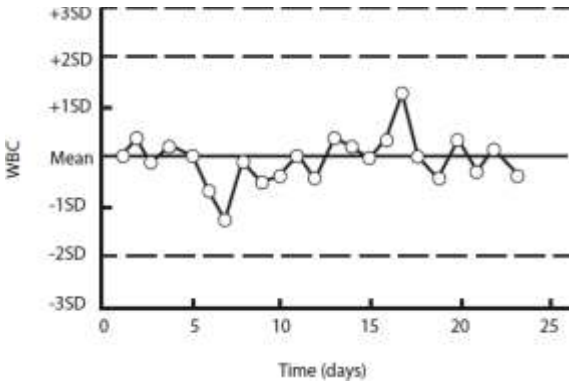
Stability is decreased if the manufacturer's recommendations for handling and storage are not followed. Also, as with reagents, evaporation and contamination are concerns. When commercial control preparations are used, either two or three "levels" of control are run. One level has values in a typical patient reference range, while the other levels have abnormally low and high values.

Retained Patient Specimens (Secondary Controls)

Ideally, control preparations should be very similar to actual patient samples. Unlike commercial controls, the "true" values for parameters of patient samples (secondary controls) are not known. However, patient samples have the advantages of being identical to routinely tested samples and less expensive than commercial controls. Patient samples are also useful to distinguish between (1) instrument or operator errors and (2) problems with commercial controls. A patient specimen whose values were determined when the instrument was "in control" may be used for a maximum of 24 hours after collection.

The RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, and MPV are stable for up to 24 hours, while the total WBC should be within 5% of its initial value for up to 12 hours, and within 7% for up to 24 hours. The WBC differential parameters are stable to within 10% of their initial values for up to 12 hours. Stability studies indicate that samples exhibit increased stability when they are stored at room temperature rather than stored in the refrigerator.

Plotting control data on chart



Using Levey-Jennings (or L-J) charts, to graph the results of control performance is suggested. Each time a control is analyzed, the result is added to the chart. We can then look at the series of dots and detect patterns of performance. For example (attached graph)

Establishing targets/ Limits

The target value adopted by individual laboratories should be established through analysis of the mean value obtained for each parameter after processing multiple replicates of the respective controls. This lab-generated mean value should fall within the manufacturer-provided range and serves as a check on control product integrity. In other words, Assay Sheets or electronically provided target values and ranges from a manufacturer are simply broad starting guidelines, but not the values for use during the life of the control material in a particular laboratory.

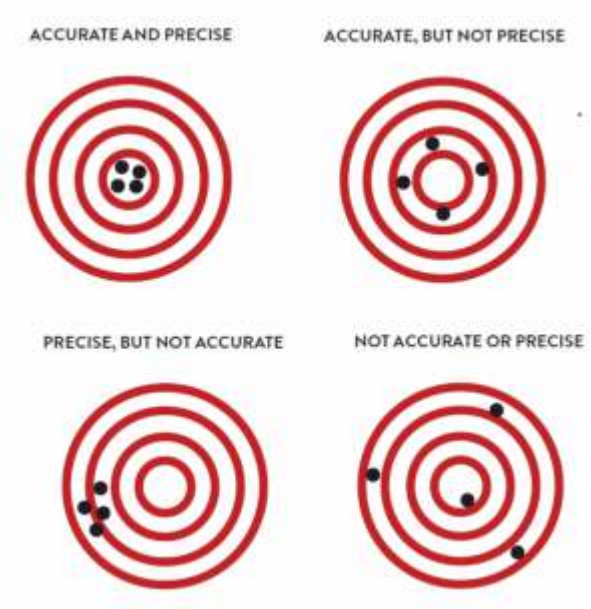
Understanding Accuracy and Precision What is Accuracy?

Accuracy is the ability to get the right answer.

Think of an archer shooting arrows at a target. The archer wants his arrow to hit the center of the target to get the best score. He loses points when he is not in the center. The farther from the center the fewer points he gets until, if he misses the target altogether, he gets no points. This is accuracy: the ability to hit the center of the target.

In the laboratory, accuracy is the ability to get the right answer, whether we are analyzing a patient's sample or a control sample. If the control sheet says that we should have a result of "x", if everything is operating properly, we would expect to get this result, or very close to it. A little bit of variation is normal and expected.

What is Precision?



Precision is the ability to get the same answer, over and over.

Consider our archer again. He does not want to hit the center of the target just once; he wants to hit it every time. His archery is said to be precise if all of the arrows are close together. In our case, we want all of the control results to be close together. As with accuracy, a little bit of variation (or, to get technical, imprecision) is normal. Your control limits will tell you how much is allowed.

Types of Hematology Analyzer

a. **3-part differential**: The differential of three parts does not divide the granulocytes into neutrophils, eosinophils, and basophils. The machine warns the user to irregular numbers of RBC or WBC, atypical lymphocytes, and giant platelets.

b. **5-part differential**: Several analyzers for hematology have 5-part differentials. The WBCs are categorized in neutrophils, lymphocytes, monocytes, eosinophils and basophils in the normal study.

Limitation of Hematology Analyzer

- Two cells passing through the orifice at the same time counted as one cell.
- RBC agglutination (clump of cells) counting bubbles.
- Counting other particles as cells.

References:

1. Learning Guide: Quality Control in The Hematology Laboratory. Corelaboratory.abbott/ hematology.
2. CLSI. Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard - Second Edition. H26-A2. Wayne, PA: CLSI, 2010.
3. Ward P.G, Wardle J., Lewis S.M. Standardization For Routine Blood Counting- The Role of Interlaboratory Trials. In Cavill I.Quality Control. Methods in Hematology volume 4 Edinburgh, UK: Churchill Livingstone, 1982;4;102-120.
4. Streichman S, Gescheidt Y. Using Patient Blood Samples to Assess and Harmonize Laboratory Test Results. Lab Hematol. 1998; 4:21-25.

ESR ANALYZER

- Quantitative Capillary Photometry studies the dynamic behavior of red blood cells (RBCs).
- The blood sample flows in a transparent capillary inside the instrument and the reactivity of the red blood cells is analyzed when this flow is suddenly interrupted: this abrupt interruption, together with the rheological characteristics of the sample itself, and the presence or absence of the proteins of the acute phase in it, starts or not the process of aggregation by stacking red blood cells.
- Follow the instrument manual for installation requirement, operation and maintenance.
- Operation training must be given by company representatives.
- Equipment engineer must provide 3 qualification certificates of instrument (IQ- installation qualification, OQ- operational qualification, PQ- performance qualification)
- Equipment calibration as per user manual of instrument
- Daily- cleaning of surface, clean spill immediately, Disposal of waste fluid, Wipe away the residual liquid on the reagent holder, sample, or reagent needle.
- Weekly cleaning- Fan, pipe cleaning
- Monthly- LED calibration
- Quarterly/ Biquarterly- all interiors, tubing, filters, calibrate instrument.

VDRL ROTOR/SHAKER

- The Venereal Disease Research Laboratory (VDRL) Rotator is an apparatus that is mostly used for Agglutination Tests, Blood grouping test and mixing of solutions in Conical Flasks, Beakers & Bottles
- VDRL rotator should be installed according to the instructions in the Manufacturer's Manual, specific to each rotor.
- Keep the rotator in a well-ventilated dry space, on a level surface.
- Ensure that the location has a suitable amount of space for putting the samples and the accessories required for the normal operation of the VDRL rotator.
- Avoid placing the rotator next to vibrating apparatus as it can interfere with its normal operation.

- Avoid cluttering of samples, ensure that there is enough placed to keep samples as well as reagents, so as to avoid spillage.
- Before putting samples ensure that the rotation is smooth and not jerky as it can cause spillage of sample
- Ensure that the rotator is kept on a level surface so that the samples do not flow off.

Figure 24: VDRL ROTOR/ SHAKER
Source: https://images.app.goo.gl/89hwNc2vYvEY2MpF6

Using the VDRL Rotator

- Ensure that the VDRL rotator is connected to the mains.
- Check the level with a spirit level.
- Wear PPE (at least apron & latex gloves) and perform the test procedure.
- Space out the VDRL tiles/ RPR cards, without overcrowding.
- In case it is a fixed rotation VDRL Rotator turn on the knob to the desired time interval
- Interpret result after the rotator has stopped.
- Avoid spillage of the sample on the platform, in case there is spillage, immediately put absorbent paper on the spillage, pour 1% hypochlorite on the paper & leave it for minimum 30 minutes. After 30 minutes, pick up the paper with gloved hands, throw the soaked paper in red bag and swab the platform with fresh 1% hypochlorite solution.

VDRL Rotator cleaning and calibration

Cleaning

- Reagents and solution: Cloth dampened with 1% hypochlorite or 70% ethanol.
- Period: Every month or in case of spillage

Procedure:

- Disconnect the plug from the main socket.
- Clean metallic surfaces with the disinfectant
- Clean the external surface with a cloth dampened with water.
- Make an entry in the maintenance log sheet.

Calibration of Rotator

- **Speed**: For rotators without a digital readout, the speed can be estimated by counting the number of rotations made per minute. To count the rotations, hold your finger next to the rotator and count the number of times the rotator touches your finger in 15 seconds. If the rotator is properly adjusted, the count should be 45. The rotator should be calibrated each day it is to be used.
- **Time:** The rotator's timer should be checked against another laboratory timer or stopwatch. The rotator's timer should be within ±15 seconds of the set time.

CENTRIFUGE

- Centrifuges are machines used in the laboratory for spinning the samples in order to separate out fractions.

- Centrifuges are potentially lethal equipment; care and vigilance need to be exercised at all times.
- Laboratory centrifuge: An apparatus used in the laboratory for separating substances of different density or particle size, when suspended in a fluid, by spinning them about an axis in a suitable container.
- Rotor: Primary component of a centrifuge which holds the material to be subjected to centrifugal force (in some form of tube/container) and which is rotated by the drive system.
- Rotors may be fixed angle or swing bucket rotors.
- RCF (relative centrifugal force) generated by a rotor depends on the speed of the rotor in revolutions per minute (rpm) and the radius of the rotation. Many centrifuges measure speed in revolutions per minute (rpm) or RCF.

Figure 25: CENTRIFUGE
Source: https://images.app.goo.gl/fDy2FxZb1n81dPN18

Formula to find RPM:

$$RPM = 1000 \times \sqrt{RCF} / 1.12 \times r$$

r = radius in mm from the centrifuge spindle to point of tube

RCF = desired centrifugal force

Safety precautions- Centrifuge

- All operators should receive training on the safe operation of the centrifuge prior to using the equipment.
- Do not centrifuge flammable or explosive materials.
- Do not exceed the maximum rated speed for any rotor.
- Always use a balance to verify even weight distribution.
- Always place the centrifuge on an even, sturdy platform
- Always use the correct tube or tube/adapter combination for the rotor.
- Stay with the centrifuge until full speed is attained. Abort the run if there is any unusual noise, vibration or smell and report immediately.
- Never attempt to open the lid when the rotor is spinning.
- Keep Biological Spill Kit near the centrifuge site. In the event of a spill inside the centrifuge, keep lid closed and refer to Biological Spill Control SOP for biohazard spills.
- Load samples and blanks into opposing rotors or baskets. Ensure the load is fully balanced – minor variations will be amplified at speed.
- If the samples are to be centrifuged at low temperatures, pre-chill the rotor and ensure the centrifuge chamber temperature is set appropriately.
- When the run is complete, wait for centrifuge to come to a complete stop before attempting to open lid. The lid is interlocked and will not function while rotor is spinning.
- On completion of running, inspect the tube for any tube breakage and leakage of sample.
- Follow spill cleaning procedures

Table 10: Centrifuge maintenance

Daily maintenance	Monthly maintenance	Annual maintenance
• Wipe the inside bowl with disinfectant solution and rinse thoroughly. For a refrigerated centrifuge that is turned off at night, open the top to allow the bowl to dry. During the day when the unit is under refrigeration, leave the top closedto avoid condensation and ice buildup. • The centrifuge must not be used if the interior is hot, if unusual vibrations or noises occur, or if deterioration (corrosionof parts) is detected. A qualified service technician should be contacted. • Most vibrations are due to improper balancing and can be corrected by re-balancing the buckets and tubes.	• Clean the centrifuge housing, rotor chamber, rotors and rotor accessories witha neutral cleaning agent. • Clean plastic and non-metal parts with a fresh solution of 5% sodium hypochlorite (bleach) mixed 1:10 with water (one part bleach plus nine parts water). • Removal of bleach must be done by wiping with 70% alcohol.	• Service is to be performed by a qualified technician. • The service technician must ensure that the unit operates safely and properly. This would include cleaning condenser coils, fans, screens, filters and checking the centrifuge brushes, bearings, timer, temperature, speed and for electricalintegrity

TOTAL RBC COUNT

Aim : To enumerate the total number of red blood cells (RBC) in blood sample.

Method : Hemocytometer (Neubauer) Counting Method

Figure 26: NUBER'S COUNTING CHAMBER
Source: https://images.app.goo.gl/KZVYowKcVyyvq- T4a6

Material Required:

1. Hemocytometer chamber (Nuber's chamber
2. Cover slip.
3. Light microscope.
4. RBC pipette.
5. RBC diluting fluid (Haeyem's solution or Physiological saline 0.85% Nacl).
6. Blood sample
7. Cotton

Procedure

1. Take RBC duluting pippte previosly cleaned with alcolohol
2. Aspirate blood sample upto 0.5 marks (Note: should aspirate above the 0.5 mark and slowly release excess blood and keep at 0.5 mark, extra care should be taken)
3. Clean excess blood at pipette tip by wiping with cotton.
4. Carefully aspirate RBC diluting soulution upto 101 mark in the pipette.
5. Hold the pippete into horizontal position.

Figure 27: RBC PIPETTE
Source: Modified Principles of Haematology LabManual-Fall 2016%20(1).pdf

6. Mix the blood into diluting solution thoroughly by shaking the pipette for 2-3 min.
7. Once the blood sample has mixed properly (dilution is 1:200).
8. Prepare counting chamber for sample loading.
9. Take counting chamber and cover slip cleaned with alcohol.
10. Carefully position the coverslip on top of the support platform located on both side of couting chamber.
11. Now take, hemolyzed sample which was in RBC diluting chamber, discard 2-4 drops of sample to avoid air bubble.
12. Carefully position tip of the pipette on the counting chamber benethe te cover glass and dispense the sample (capilary action will take the sample to spread on the counter).
13. Allow other counting chamber in same way for sample loading on the counter.
14. Once loading done, keep aside counting chamber to allow RBCs to sattle.
15. Slide is now ready for microscopy.

Microscopy

1. Place conting chamber on the microscope platform
2. First set microscope at low power (10X) to see squares at the centre of the microscope
3. Focus on middle most square for RBC counting
4. Once located RBC counting squares, change 40X objective
5. Locate the fisrt 5 (1^{st}, 5^{th}, 13^{th}, $21^{st}$and 25^{th}) smaller squares in the RBC counting chamber
6. Rule: L line (means count RBC found on the centre found on left and lower line of the square), do not count cells lying on upper and right side border of the squares.
7. Count the number of cells in 5 quares as given above

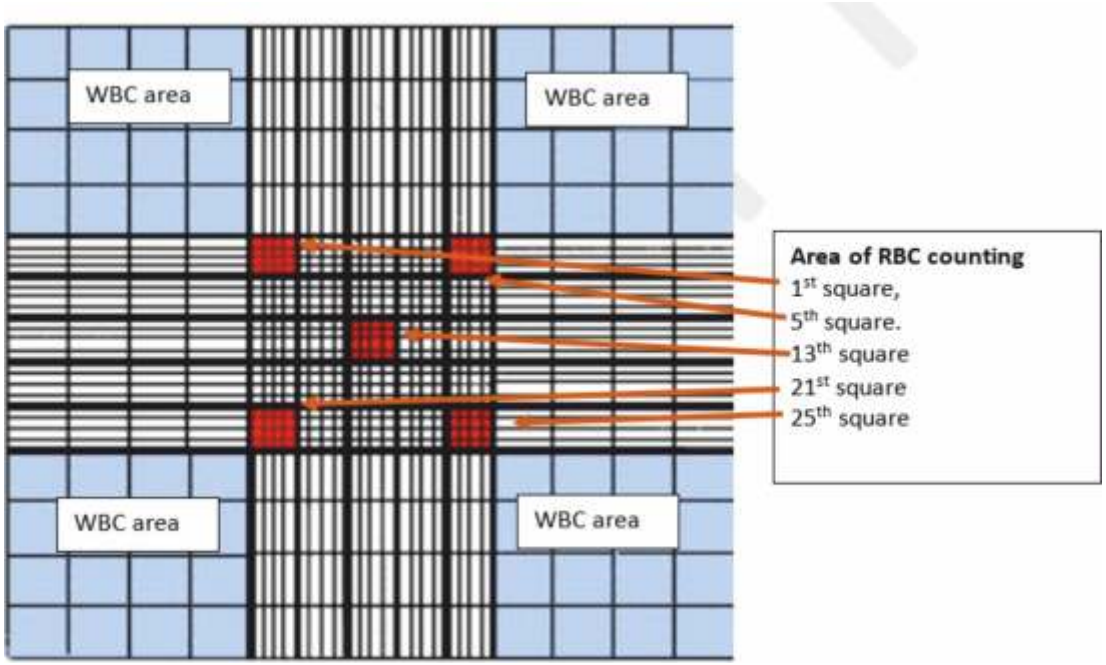


Figure 28: CELL COUNTING GRID
Source: ModifiedPrinciplesofHaematologyLabManual-Fall2016%20(1).pdf

Formula: Shortcut method

Total RBC Count per cu mm (per microlitre) = Sum of number of cells in 5 squares (1^{st}, 5^{th},13^{th}, 21^{st} and 25^{th}) X10,000

OR

Total RBC Count per cu mm (per microlitre)= (No. of cells counted X dilution factor X depth factor)/ Area counted)

In this case : Dlution factor- 200, depth factor is 10, Area counted is 80/400= 1/5 sq mm, No of cells is N

Then, RBC count = N X 200 X 10 X 1/5 RBC count= NX 10,000

Normal RBC count in male = 4.6 to 6.1 million cells/ cu.mm

Normal RBC count in Female = 4.2 to 5.4 million cells/ cu.mm

TOTAL LEUKOCYTE COUNT

Aim : To enumerate the total number of red blood cells (RBC) in blood sample.

Method : Hemocytometer (Neubauer) Counting Method

Material Required:

1. Hemocytometer chamber (Nuber's chamber).
2. Cover slip.
3. Light microscope.
4. WBC pipette.
5. WBC diluting fluid (1% HCl or 1% Glacial Acetic Acid) [1ml GAA+1ml MB +100ml DW).
6. Blood sample
7. Cotton

Procedure

1. Take WBC duluting pippte previosly cleaned with alcolohol
2. Aspirate blood sample upto 0.5 marks (Note: should aspirate above the 0.5 mark and slowly release excess blood and keep at 0.5 mark, extra care should be taken)
3. Clean excess blood at pipette tip by wiping with cotton
4. Carefully aspirate WBC diluting soulution upto 11 mark in the pipette

Figure 29: COUNTING CHAMBER
Source: ModifiedPrinciplesofHaematologyLabManu - al-Fall2016%20(1).pdf

5. Hold the pippete into horizontal position
6. Mix the blood into duluting solution thoroughly by shaking the pipette for 2-3 minutes.
7. Once the blood sample has mixed properly (dilution is 1:20)
8. Prepare counting chamber for sample loading
9. Take counting chamber and cover slip cleaned with alcohol
10. Carefully position the coverslip on top of the support platform locted on both side of couting chamber.

11. Now take, hemolyzed sample whichh was in WBC diluting chamber, discard 2-4 drops of sample to avoid air bubble.

12. Carefully position tip of the pipette on the counting chamber benethe te cover glass and dispense the sample (capilary action will take the sample to spread on the conunter)

13. Allow other counting chamber in same way for sample loading on the counter

Figure 30: WBC PIPETTE

14. Once loading done, keep aside couting chamber to allow WBCs to sattle.

15. Slide is now raedy for microscopy

Microscopy

1. Place conting cahmber on the microscope platform
2. First set microscope at low power (10X) to see squres at the centre of the microscope
3. Focus on squares for WBC counting
4. Once located WBC counting squares, change 40X objective
5. Locate the 4 WBC couting squares
6. Rule: L line (maens count WBC found on the centre found on left and lower line of the square), do not count cells lying on upper and right side border of the squares.
7. Count the number of cells in 4 squares as given above

Calculations

WBC/µL or cu mm = No. of cells in 4 squares (64 small squares)/
4 x dilution factor. x reciprocal of chamber depth.

In this case: dilution factor is 20, depth of chamber is 1/ 10 mm,

So, number of WBC per microliter = N/4X20X10

WBC/µL= No. x 50

Possible errors in Total Erythrocyte and Leukocyte Counting

1. Errors in dilution, counting, calculation and calibration.
2. Improper shaking of the pipette after dilution.
3. Contamination of diluting fluids with yeast, mold,or other cells.
4. Failure to wipe excess blood from the end of the pipette.
5. Drying of the sample during or prior to counting.
6. Overflow of fluid into the moat.
7. Failure to allow the cells to settle into a single plane prior to counting.
8. Failure to discharge diluting fluid from the capillary prior to charging the chamber.
9. Chipped pipettes.
10. Inadequate cleaning of the glass wares.
11. Failure to focus microscope up and down during counting.

TOTAL PLATELETS COUNT

- Platelets or thrombocytes are the smallest blood cells that are derived from megakaryocytes cytoplasmic fragmentation in the bone marrow.
- Platelets are found only in mammals as small fragments between the red cells containing small purple-staining granules. -EDTA anticoagulant minimizes platelet clumping.
- They have a tendency to extrude hair like filaments from their membranes especially during activation.

Morphology of Thrombocytes

- They are anucleate (incapable of cell division or mitosis like RBC).
- They may be round, discoid, flat, spheroid, elongated, oval or rod shaped.
- The cytoplasm stained light blue hyalomere (clear) with red or purple granules.

Thrombocyte Counting

1. Manually by using a hemocytometer.
2. Automatically by using platelet analyzer (Coulter Counter).

Hemocytometery Method

- To investigate abnormal skin and mucosal bleeding which can occur when the platelet count is very low.
- Platelet counts are also performed when patients are being treated with cytotoxic drugs or other drugs which may cause thrombocytopenia.

Materials required

- Hemocytometer counting chamber.
- 1% Ammoium oxalate to hemolyzed erythrocytes.
- EDTA anticoagulated venous blood.
- Capillary blood should not be used because platelets clump as the blood is being collected.

Procedure

1. Pipette 990µl of 1% ammonium oxalate into a clean, dry test tube.
2. Add 10µl well mixed venous blood.
3. Leave at room temperature for 10 minutes to allow the platelets to settle down.
   a. Use micropipette tip to take 20 µl from the prepared solution and the tip is touched to the side of the hemocytometer chamber (without any hesitation) till a fluid will run under the cover glass.
4. Mount the hemacytometer on the microscope and total number of cells in 1 large square on a corner (25 small squares) is determined under the high dry objective power (40X).
5. To avoid duplicate counting of a single cell, you must count only those cells that touch the lower and left boundaries. Platelets appear greenish, not refractile.

Calculations

Total number of cells counted in one square X Reciprocal of dilution.

Cells/ Cu mm = Number of squares counted X Area of each square X Depth of the solution.

Example:

Total no of cells = N, Dilution= 1: 100, No of square counted= 1, Area of each square= 1 mm cu, Depth of solution= 0.1 mm

Then, Cells/ Cm mm = N X 100/ 1 x 1 mm cu X 0.1 mm

= N X 1000/ cub mm (micro liter) or N X 10^3 per/ L Normal range.

The normal number of platelets in the blood is 150,000 - 400,000 platelets/µl

HEMOGLOBIN DETERMINATION

Aim : To determine the amount of Hb present in 100 ml of blood of a given sample.

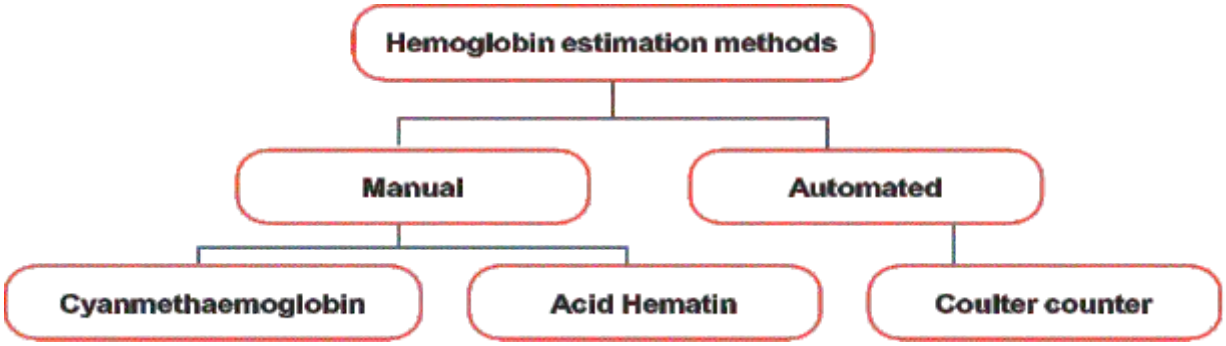
Methods of Hemoglobin determination

3.8.1 Acid Hematin Method

Material required**:** Sahli set.

Parts of Sahli Set

1. Sahli standard (color comparator) for matching color.
2. Hydrochloric acid (HCl 0.1N) for hemolyzing of RBC and converting blood to acid hematin.
3. Graduated hemometer tube.
4. Hemometer or Sahli's pipette with rubber tube and plastic mouthpiece for sucking of the blood.
5. Glass rod or stirring rod for mixing.
6. Small brush for cleaning.
7. Dropper for HCl suction.



Reagents

1. Need distilled water (DW) for diluting acid hematin and matching to the standard color.
2. 0.1 N HCl is prepared by mixing 0.8 mL concentrated HCl with 99.2 mL DW.

Procedure

1. Add 0.1 N hydrochloric acid (HCl) to the mark 2 of graduated hemoglobin tube. Take blood sample into capillary pipette to the 20 mm 3mark with well mixed, un clotted sample by gentle suction.
2. Wipe off all the blood adhering to the outer surface of the pipette with cotton wool.
3. Expel the blood sample into the acid solution in the graduated tube.

Figure 31: PART OF SAHLI SET
Source: https://images.app.goo.gl /arKEpcYUgZDE8Nsn7

4. Rinse out the pipette with the solution and thoroughly mix the tube contents with glass stirrer.
5. Stand it for 5-10 min for color development.
6. Dilute it with DW, by adding drop by drop and mixing thoroughly at each addition, until the color matches that of the standard
7. Take the readings of the lower meniscus from the graduated tube.
8. The resulting brownish-yellow mixture is matched with a standard in a colorimeter, and from this determines the hemoglobin content of the blood.

Figure 32: SAHLI'S HEMOGLOBINOMETER
Source: https://images.app.goo.gl/N5gTQadrUuhcsa8q6

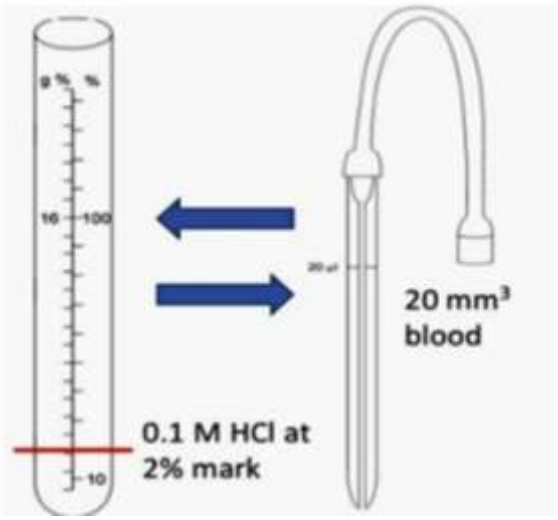


Figure 33: Hemoglobin Tube and Pipette
Source: https://images.app.goo.gl/GYojEhqDec7e8cEz5

Sources of Error

1. Non hemoglobin substances normally present in the plasma, such as protein and lipids.
2. Usually, it is difficult to match the color of the sample accurately to the brown color of the standard.
3. The variation in the ability of individual operators in matching colors is the common source of error.
4. Some inactive forms of Hb present in the blood, such as methemoglobin, Sul hemoglobin and carboxyhemoglobin, which are not converted in acid solutions into hematin, are not included in values obtained by this technique.
5. Improper mixing of blood in the comparator.
6. Pipetting error during blood suction.

PACKED CELL VOLUME (PCV) DETERMINATION

The Packed Cell Volume (PCV) test, also known as the Hematocrit (HCT) test or Erythrocyte Volume Fraction (EVF). PCV is the volume percentage (%) of erythrocytes in the blood.

Aim : To determine the hematocrit value in the given blood sample

Principal **:** Blood sample is separated into three parts using capillary tube in a hematocrit centrifuge.

Significance:

- Packed Cell Volume (PCV) = erythrocyte mass; anemia when PCV falls.
- Buffy coat; white to gray layer above PCV. It will give the number of WBC (0.5mm to1.5mm).

Leukopenia or leukocytosis.

- Plasma content: usually about 55%, Yellowish in color. The degree of yellowness indicates icterus (jaundice).

Methods of testing

1. Microhematocrit (capillary tube) method.
2. Macrohematocrit (wintrobe) method.
3. Automated estimation of PCV.

Microhematocrit (capillary tube) method. Requirement

1. Capillary tube (Microhematocrit tube) with anticoagulant (red tip).
2. Special clay or sealing clay.
3. Microhematocrit chart (Reader).
4. Hematocrit centrifuge

Figure 34: Special Wax/ Clay For Capillary Tube Sealing
Source: ModifiedPrinciplesofHaematology LabManu- al-Fall2016%20(1).pdf

Figure 35: Hematocrit Centrifuge
Source: ModifiedPrinciplesof Haematology LabManu- al-Fall2016%20(1).pdf

Figure 36: Hematocrit Chart
Source: ModifiedPrinciplesofHaematology LabManu- al-Fall2016%20(1).pdf

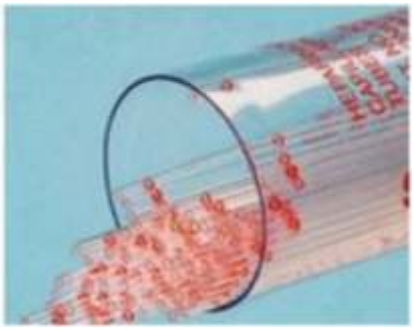


Figure 37: Capillary Tube
Source: ModifiedPrinciplesofHaematology LabManu- al-Fall2016%20(1).pdf

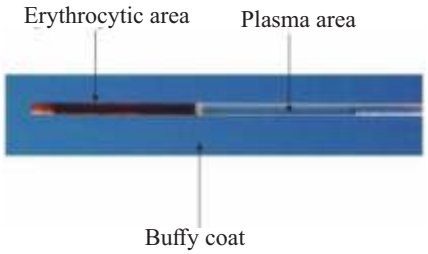
Procedure:

1. Take blood in capillary tube about ¾ filled.
2. Clean and dry the outside of the tube carefully with a piece of gauze.
3. Seal the opposite end of the tube with special clay/ wax or by heating in a flame.
4. Place the sealed tubes in a hematocrit centrifuge, replace the cover, tighten securely, and run for 4 minutes at 10,000 rounds per minute (RPM).

5. **Note:** The sealed end should face the outside rim of the centrifuge to avoid the breakage of the tube. You have to use 2 tubes at the same time in opposite directions to balance the centrifuge.

6. After centrifugation, carefully place the tube on a special microhematocrit reader for determining the percentage of RBC.

Separated layers:



After centrifugation, blood is separated into 3 distinct parts.

a. The mass of erythrocytes at the bottom (packed cell volume).

b. Grey or white layer of leukocytes and thrombocytes immediately above the red cell mass (Buffy coat).

c. The blood plasma (slight yellow color).

Sealing of capillary tube over flame (empty end)

Or sealing with special wax/ clay (empty end)

Figure 38: Reading On Microhematocrit
Source: ModifiedPrinciplesofHaematologyLabManual-Fall2016%20(1).pdf

Normal Values

- Male: 40-52%
- Female: 36-48%

Note:

- Venous blood samples must be collected in suitable anticoagulant.
- Recommended anticoagulant is EDTA, but care must be taken not to use it in excess, as the PCV will be decreased.

Sources of Errors

a. Hemolyzed blood.

b. Improperly Shaked venous blood.

c. Inadequate volume of blood.

d. Improper sealing.

e. Not enough centrifugation and separating of layers.

RED CELL INDICES OR ERYTHROCYTE INDICES

- This test provides information about the Hb content and size of red blood cells.
- Abnormal values indicate the presence of anemia and which type of anemia it is.

Aim: to determine or classify different types of anemia

Objectives

- To be able to calculate MCV, MCH, and MCHC from red blood cell count, hematocrit, and hemoglobin concentration.
- To compare normal ranges of the blood indices of different domestic animals.

Significance

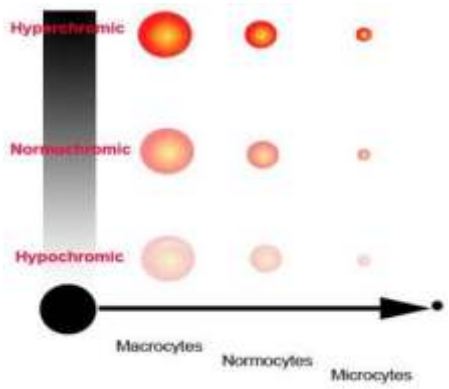
- Here one can classify red blood cells as normo, hypo and hyperchromic anemia.
- Apart from these facts, the size of red blood cells as normo, micro and macrocytosis can be classified.

Erythrocyte Indices Tests

1. Mean corpuscular volume (MCV)
2. Mean corpuscular hemoglobin (MCH)
3. Mean Corpuscular Hemoglobin Concentration (MCHC)

Mean corpuscular volume (MCV)

- It expresses the average size of the RBC.
- The calculation is as, **MCV= (PCV/RBC) X 10** in fl (femtolitre)
- Normal range is 80 to 100 fl
- Interpretation: Normocyte, Microcyte and Macrocyte



Mean corpuscular Hemoglobin (MCH)

- It is the average amount of Hb per red blood cell.
- The calculation is as, **MCH= (Hb/RBC) X 10**
- The result is expressed in picogram (pg).
- Normal range: 2 to 31 pg/cell
- Interpretation: normochromic, hypochromic, hyperchromic

Mean Corpuscular Hemoglobin Concentration (MCHC)

- It is the average concentration of Hb relative to volume of red blood cells.
- The calculation as, MCHC= **(Hb/ PCV) X 100**
- Expressed in gm/dl
- Normal range is 32 to 36 gm/dl
- Interpretation: normochromic, hypochromic, hyperchromic

Parameter	Definition	Unit	Formula
Mean Corpuscular Volume (MCV)	Average volume of the RBC	Femtoliters (fL) or 10-^{15}Liters	**MCV=** Hematocrit (%) X10 / RBC (x 10^{12})/ L
Mean Corpuscular Hemoglobin (MCH)	Average weight of Hb in the RBC	Picograms (pg) or 10-12 grams	**MCH =** Hb (gm/dl) X10 / RBC (x 10^{12})/ L
Mean Corpuscular Hemoglobin concentration (MCHC)	Average concentration of Hbin the RBC volume	Grams/deciliter (g/dL)	**MCHC =** Hb (gm/dl) X10 / Hematocrit (%)

ERYTHROCYTE SEDIMENTATION RATE (ESR)

Aim: To determine the rate at which RBCs are falling (regimenting) during a given period of time.

Principal

The distance that the erythrocytes fall during a given period when blood to which anticoagulant has been added in a tube placed in a vertical position.

Significance

- It is not a specific test but reflects the change in plasma protein accompanying most of acute and chronic infection.
- Some pathological conditions cause rouleax formation.
- The greater the ESR reading, the more the severity of pathological condition.
- During TB and rheumatic disease ESR increases drastically.

Methods: can performed by two methods

1. Manual (Westergren or Wintrobe method).
2. Automatically (ESR analyzer).

Wintrobe Method

Material required.

Wintrobe set which composed of:

1. Wintrobe tube (pipette), calibrated in mm.
2. Wintrobe rack.
3. Timer required.

Procedure

1. Mixed venous blood containing anticoagulant is filled into a Wintrobe tube.
2. Put the tube in a sedimentation rack (Westergren) that holds it perpendicular to the surface of the tube.
3. The rack should be placed in an isolated area in the laboratory that is free from vibrations.
4. The filled tube in a rack is allowed to stand for exactly 1 hour.
5. Lastly the level of the top of the erythrocyte column is recorded as the number of millimeters of fall per hour (mL/hr).

Figure 39: Wintrobe Tube
Source: ModifiedPrinciplesofHaematologyLabManu- al-Fall2016%20(1).pdf

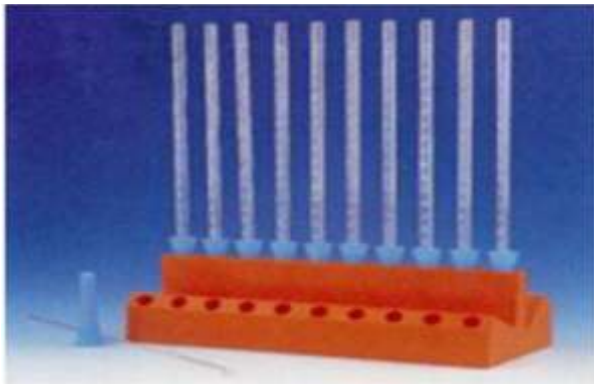


Figure 40: Wintrobe Rack
Source: ModifiedPrinciplesofHaematologyLabManu- al-Fall2016%20(1).pdf

Note:

Figure 40: Timer
Source: ModifiedPrinciplesofHaematology LabManu- al-Fall2016%20(1).pdf

- Care must be taken to ensure that a standard amount of anticoagulant was used, as high anticoagulant makes erythrocytes sink faster because they become heavier than normal.
- Since temperature may influence ESR, room temperature should be near 20ºC.
- This process depends to some extent on plasma viscosity that alters when inflammatory proteins are present.
- The speed with which erythrocytes fall in the blood of normal individuals is relatively slow, but in those with inflammatory diseases in which there is tissue necrosis and degeneration; the speed is increased, which may be due to the activation of coagulative cascades and changes that occur in the physiochemical properties of the erythrocyte surfaces and the plasma.

Sources of Error

- Incorrect use of anticoagulants.
- Presence of substances such as dirt, alcohol or ether within the Wintrobe tube.
- Not placing the tube vertical to the table.
- The presence of air babbles in the tube.
- Hemolysis of the blood sample.
- Refrigeration of blood samples.
- Old blood sample.

PERIPHERAL BLOOD SMEAR MICROCOPY

Blood smear determines the exact number and/or the percentage of each type of white blood cell that is present in an individual stained blood smear.

Blood smear Preparation (Thin smear)

1. It is mainly useful for detection of cell morphology and differential cell count.
2. Place a drop of blood on the end of a clean glass slide using hematocrit tube.
3. Hold another slide (or cover slip) firmly in a horizontal position (an angle of about 30-45^0) just in front of a drop of blood.
4. Pull the horizontal slide somewhat backward in order to make the drop of blood run along the line of contact between the 2 slides.
5. Push the horizontal slide gently but firmly along the surface of the first slide to the far end.
6. Dry rapidly by waving in the air or using a blower.
7. Refer to video link https://www.cdc.gov/dpdx/images/diagnosticProcedures/Thin2.mp4

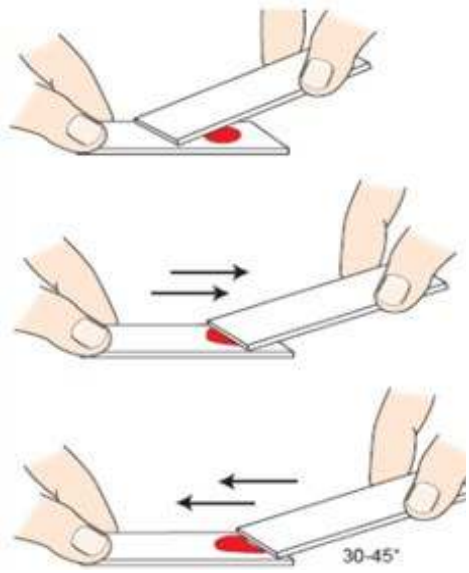


Figure 41: Thin Smear Preparation
Source: https://images.app.goo.gl/39ouos8DgKE1VxWH6

Blood smear Preparation (Thick smear)

- It is most useful for detecting blood parasites (especially malaria), because they examine a larger sample of blood, even if few numbers of parasites are available in the blood at the time the test.
- Additionally, the percentage of red blood cells that are infected (parasite density) and what type of parasites are present can be detected.
- Refer to video for thick smear preparation: https://www.cdc.gov/dpdx/images/ diagnostic Procedures /Thick1.mp4

Criteria of Good blood smear (Thin)

1. Smooth and an even appearance.
2. The smear has long straight borders.
3. The RBCs are distributed in a single layer in a major part of the smear.

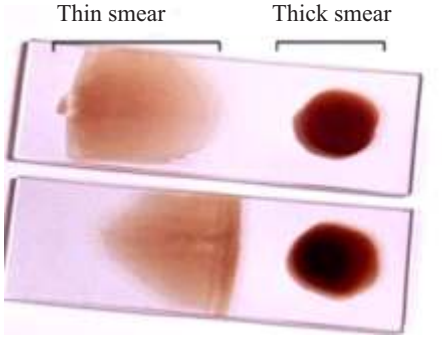


Figure 42: Thick Smear Preparation
Source: https://images.app.goo.gl/81NzRwD8GJYosDeH9

Types of stain for peripheral blood smear

Wright Stain: It is a mixture of eosin (red) and methylene blue dyes, used primarily to stain blood and bone marrow aspirate smears.

Giemsa Stain: It is used in cytogenetics and for the histopathological diagnosis of malaria. Giemsa's solution is a mixture of methylene blue, eosin, and Azure B.

Wright-Giemsa Stain: It is a combination of Wright and Giemsa stains.

May-Grünwald Stain: It produces a more intense coloration, and also takes a longer time to perform. May-Grünwald and Giemsa are stable for five years. The bottles must be kept closed. The advised storage temperature is 18 - 30°C.

Leishman Stain: It provides excellent staining quality. It is generally used to differentiate and identify leukocytes, malaria parasites, and trypanosomes.

Fixing the film

- To preserve the morphology of the cells, films must be fixed as soon as possible after they have dried.
- It is important to prevent contact with water before fixation is complete.
- Methyl alcohol (methanol) is the choice although ethyl alcohol can be used.
- Methylated spirit must not be used as it contains water.
- To fix the film place then in covered staining jar or tray containing alcohol for 2-3 minutes. In humid climates it might be necessary to replace the methanol 2-3 times per day.

Giemsa staining procedure.

1. Immerse the blood film in absolute methanol (99%) for 5 minutes to fix the film.
2. Drain the methanol and stand the slide upright to dry.
3. Immerse the slide in working solution (1: 10) for 15 minutes or (1:20) for 30 minutes.
4. Wash the slide with D.W. to remove excess stain.
5. Place it in an upright position to dry completely.
6. Mount with DPX and put cover slip.
7. Examine the slide under oil immersion lens (HPF, 100X).

General reasons of poor staining

1. Buffer and tap water are not at proper pH.
2. Improper stain (wrong preparation and filtration).
3. Too thick smear.
4. Insufficient washing.
5. Too long fixation period.
6. Excessive staining time.
7. Not enough stains were placed on the slide.

General Cautions during Staining

1. The buffer must be of proper pH in order to provide the best differential staining (Buffer that is too alkaline causing the cells to become too blue, while too acidic buffer leads to little or no staining the nuclei of the cell).
2. Avoid excessive washing of the stained film as it will decolorize the film.
3. Do not dry films in an incubator or by heat as it will distort the film.
4. The air drying of the film should be done in a horizontal position.
5. Both Wright and Giemsa stains are flammable; keep away from heat, sparks, or flames.

Microscopy procedure

- Inspect the smear under low power to note distribution of cells and select an area where the cells do not overlap.
- Switch to oil immersion objective for further examination.
- Fields selected for examination should be those in which erythrocytes are well separated and the leukocytes thinly spread.
- Meander system should be carried out as it avoids recounting of the same field.
- Avoid fields in which erythrocytes are stacked up or aggregated.
- Enumerate at least 100 leukocytes and classify them according to their staining reactions, nuclear morphology and the characterization of any cytoplasmic granules that may occur.
- Tabulate the individual cells in columns on a prepared sheet of paper.
- Express the values of each cell as a percentage.

Table 9: Cell Appearance after staining

Type of cell	Appearance in color
Erythrocytes	Pink Tan
Leukocyte nuclear chromatin	Stains magenta
Lymphocytes	Clear sky-blue cytoplasm with red purple granules
Monocytes	Mosaic of pink and pale blue cytoplasm with azure granules
Neutrophils	Light purplish-pink or lavender granules in cytoplasm.
Eosinophils	Bright red or reddish-orange granules in cytoplasm
Basophils	Deep purple and violet, black granules in cytoplasm.
Platelets	Clearly demarcated red-purple granules in light blue cytoplasm.

Leukocyte Types and appearance

The circulating blood inside the vascular system is composed of plasma and blood cells. The blood cells are RBCs, WBCs and platelets. Normal WBCs are divided into: polymorphonuclear leukocytes (or granulocytes) and mononuclear cells.

1. Polymorphonuclear Leukocytes

a. Neutrophils

Mature neutrophil: Mature neutrophils have either a monolobular nucleus or its nucleus may have up to 5 lobes that are joined by thin strands giving it a segmented appearance. In properly stained blood film, neutrophils have faintly acidophilic cytoplasmic granules with deeply basophilic nucleus.

Band Neutrophil: Absent or very low in normal peripheral blood. Its cytoplasm is less granular than mature form and nucleus is sausage-shaped, kidney, bean or coiled with no lobulation (indentation).

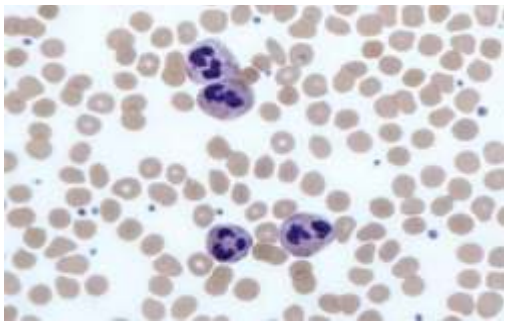


FIGURE 43: MATURE NEUTROPHIL
orange-reddish granules in the cytoplasm which may cause the cell to bulge. The nucleus is frequently non segmented, and the cytoplasm

b. Eosinophil

It is readily recognized in stained blood smears by the presence of numerous, small, circular, is faint or sky blue which may have vacuoles. Their number in circulation fluctuates throughout the day, seasonally, and during menstruation.

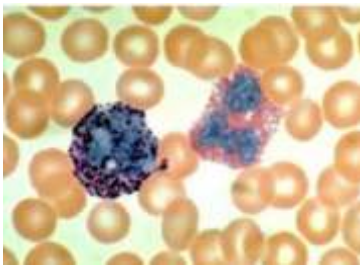


Figure 13: Eosinophil

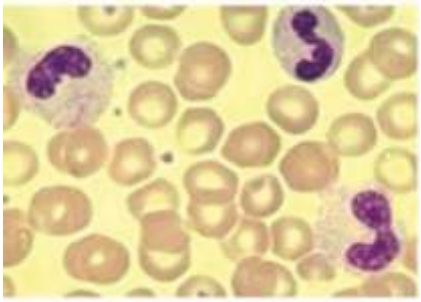


FIGURE 44: BAND NEUTROPHIL

It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system.

c. Basophil

Rarely seen in normal peripheral blood smear. It occurs only rarely in the blood of the dog and cat but most commonly seen in horses. It characteristically has purplish staining granules that scattered throughout the cytoplasm and over the surface of the nucleus.

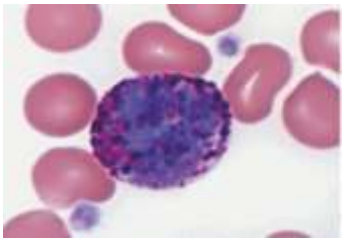


Figure 45: Basophils

<u>1. Mono nuclear Lekcocyte</u>

a. Lymphocytes

Small lymphocytes: It is the commonest form that has a small size (less than 10 µm in diameter) with large, almost circular or slightly indented nucleus and a narrow peripheral zone of blue-stained cytoplasm. The cytoplasm may contain large, dark- blue or red (azurophilic) granules.

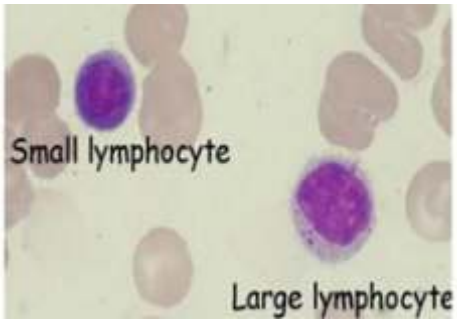


Figure 46: Lymphocytes

Large Lymphocytes: It has proportionally more cytoplasm, which stains pale-blue and may contain small vacuoles, thus causing some confusion with monocytes.

b. Monocytes

It is the largest cell in the leukocyte series. In stained blood smears, their nuclei are more varied morphologically, being oval, elliptical, kidney

or horseshoe shaped or even segmented. The cytoplasm is faintly granular, stains basophilic and may have a vacuolated or foamy appearance.

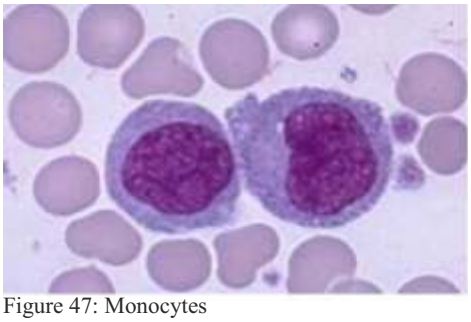


Figure 47: Monocytes

Normal WBC Values in Human

- Neutrophil: 45% to 65%
- Band neutrophil: 0% to 3%
- Eosinophil: 1% to 4%
- Basophil: 0.5% to 1%
- Lymphocyte: 20% to 40%
- Monocyte: 2% to 8%

RETICULOCYTE COUNT

- Reticulocytes are immature red blood cells without nucleus.
- They develop and mature in the bone marrow, then circulate for about a day in the blood stream before developing into mature RBC.
- Reticulocytes appear slightly bluer and larger than other red cells when looked at with the normal Romanowsky stain.
- They are called reticulocytes because of a reticular (mesh-like) network of ribosomal RNA.

Aims:

1. To determine the functioning of bone marrow and responding adequately to the body's need.
2. To help detect and distinguish between different types of anemia.
3. To monitor response to treatment (such as that for iron-deficiency anemia).
4. To monitor bone marrow function following treatments (such as chemotherapy).
5. To monitor function following a bone marrow transplant.

Method

New Methylene blue staining

Materials required

New Methylene Blue Stain (NMB)

- It is a supra vital stain that is used for the staining of immature red blood cells (reticulocytes).
- The stain makes precipitation of residual ribosomal RNA within the reticulocytes.
- As a result, the RNA appears as a reticulum within the stained reticulocytes.

Procedure

1. Take equal volumes of well mixed anticoagulated blood (usually 100µL from each) and NMB into a small vial (this allows the reticulocytes adequate time to take up the stain).
2. Mix well and incubate at 37°C for 5 minutes in a water bath.
3. Make a smear and air dry.
4. Mount with DPX, cover slip and view.
5. **Note:** NMB is toxic. Skin contact or inhalation should be avoided.

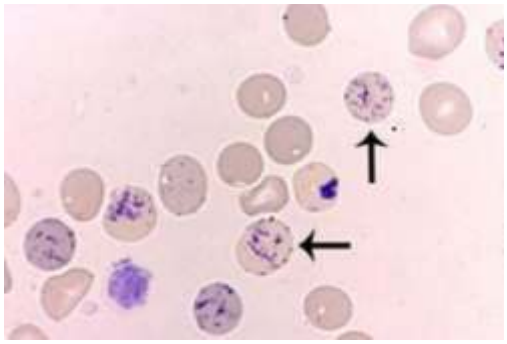


Figure 48: Reticulocyte

Staining Results

- Normal erythrocytes: stain light greenish blue.
- Reticulocytes: stain deep blue and are sharply outlined.

Calculations

Reticulocyte % = [Number of Reticulocytes ÷Number of total RBC] X 100

Normal Values

Normal healthy young adults, reticulocytes comprise 0.3 - 1% of the total number of erythrocytes.

- In young infants, the percentage is 2 - 4 times higher than for young adults.

Sources of Error

- Equal volumes of blood and stain give optimum staining conditions. An excess of blood causes the reticulum to under stain. An excess of stain usually obscures the reticulum.
- Crenated erythrocytes and rouleaux formation make an accurate count difficult to perform.
- Stain precipitated on erythrocytes causes them to appear as reticulocytes.
- The dye solution should have adequate time to penetrate the cell and stain the reticulum

COOMB'S TEST (RBC HEMOLYSIS TEST)

- The Coombs test is a set of clinical blood tests used in immunohematology to detect specific antibodies responsible for causing autoimmune hemolysis of red blood cells (RBCs).
- This test may be used to screen the blood before a procedure, such as a blood transfusion Or autoimmune hemolytic anemia.
- There are two types of coomb's test (Direct and Indirect)

Direct Coombs test (Direct Antiglobulin Test, DAT):

- This test is performed on a patient's blood sample to detect antibodies or complement proteins that are already attached to the surface of the patient's red blood cells.
- It is primarily used to diagnose autoimmune hemolytic anemia and hemolytic disease of the newborn (HDN).
- The principle of DAT is to detect the presence of antibodies attached directly to the RBCs, which takes place by washing a collected blood sample in saline to isolate the patient's RBCs; this procedure removes unbound antibodies that may otherwise confound the result.

Procedure: First step is to prepare 5% cell suspension

5% cell suspension preparation

Requirement: Blood sample, Saline, Test tubes 75x12 mm, AHGS (anti human globulin serum)

Procedure

1. Place 0.2 -0.5 ml of blood into the tube (2-3 drops).
2. Fill the tube with saline.
3. Centrifuge at 200 G for 1-2 minutes until the RBC's are packed. (G=relative centrifugal force)
4. Decant the supernatant.
5. Tap the tube to resuspend the RBC's in the residual fluid. This constitutes one wash. Repeat step 2-5 at least twice. The last wash should always have a clear supernatant with no signs of hemolysis.

6. To make a 5% cell suspension add **1 volume of the packed RBC's to 19 volumes of saline.**

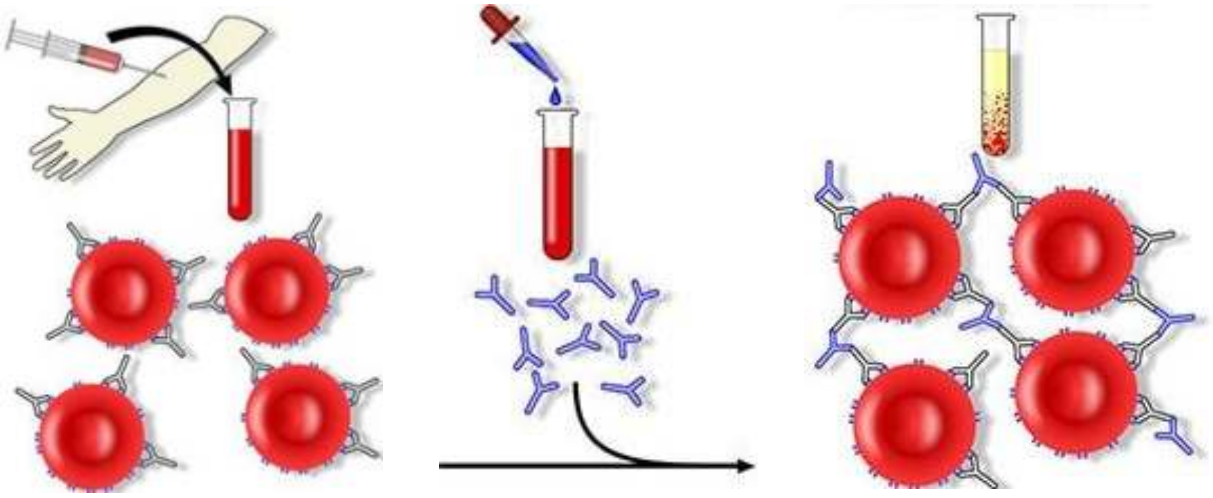
Direct Coomb's teat

Procedure

1. Take one drop of 5 % cell suspension.
2. Add two drop of anti-human Immunoglobulin serum (AHGS)
3. Centrifuge for 15 seconds
4. Read the agglutination under the microscope.

Indirect Coombs test also called indirect antiglobulin test (IAT)

- This test is performed on a patient's serum to detect antibodies that are circulating freely in the blood and potentially capable of causing destruction of red blood cells.
- It is commonly used in blood typing and cross- matching before blood transfusions, as well as in prenatal testing to assess the risk of HDN.



Blood sample from a patient with immune modified anemia: Antibodies are shown attached to antigens on the RBC surface

The patient's washed RBCs are Incubated with anti human antibodies (Coomb's reagent)

RBC's agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs

Figure 49: Concept Of Coomb's Test (Google Image)
Source: https://images.app.goo.gl/wu86obrEbqTDgQPt7

- This test is performed to detect presence of Rh- antibodies or other antibodies in patient's serum in case of following:
    - o To check whether Rh-negative women (married with Rh positive husband) had developed anti Rh antibody.
    - o Anti D may be produced in the blood of any Rh-negative person by exposure to D antigen possibly by
        - § Transfusion of Rh-positive blood
        - § Pregnancy, if infant is Rh positive (if father is Rh positive and mother Rh negative)
        - § Abortion of Rh-positive fetus

Requirements:

§ Testtube, Pasture pipettes, Incubator, Centrifuge

§ Sample: Serum

§ Reagents: Antihuman Serum, Anti-D Serum

Additional Requirement:

§ Coombs Control cells

§ Make a pool of "O" Rho (D) positive cells from at least three O positive blood samples.

§ Wash these cells three time in a normal saline (these cells should be free from serum with no free antibodies)

Procedure (Indirect Coomb' test)

1. Label three test tubes as "T" (test serum), "PC" (positive control) and "NC" negative control
2. In the tube labeled as T (Test), take 2 drops of test serum.
3. Add two drops of Anti-D serum in the test tube labeled as "PC."
4. In the test tube labeled as NC (Negative control), take 1 drop of normal saline.
5. Add one drop of 5 % saline suspension of the pooled 'O' Rho (D) positive cells in each tube.
6. Incubate all the three tubes for one hour at 37°C.
7. Wash the cells three times in normal saline to remove excess serum with no free antibodies, (in the case of inadequate washings of the red cells, negative results may be obtained).
8. Add two drops of Coombs serum (anti human serum) to each tube.
9. Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.
10. Resuspend the cells and examine macroscopically as well as microscopically.

Test Interpretation- Indirect Coomb's test		
Positive control (PC)	Agglutination	Correct overall procedure
	No agglutination	Incorrect procedure- repeat test
Negative control (NC)	Should be negative for agglutination	
Test Serum (T)	Agglutination (and if PC results are correct)	Patients Serum contains Anti-D
	No agglutination (if PC results are correct)	No Anti D in patients' serum

Coomb's tests Interpretation

	Direct Coomb's test	Indirect Coomb's test
Negative result	Antibodies not attached to red blood cells	Antibodies do not present in serum
Positive result	Antibodies attached to red blood cells	Antibodies present in serum
Used for	Hemolytic anemia	Screening of blood compatibility

BLEEDING TIME (BT)

The bleeding time is the time period measured between an initial small skin incision and the moment the subsequent bleeding stops.

Requirement

- 70% isopropyl alcohol and gauze swabs, Stopwatch, Baumanometer, Filter paper Adhesive plaster Gloves.
- Bleeding Time Device: Lancet eg Surgicutt for adult, Lancet eg Surgicutt Jr (for Children 5 months - 15 years)

Note: Avoid surface veins, scars and bruises. The arm chosen should not be paralyzed or oedematose and should not contain an intravenous line or shunt. Training module for Laboratory personnel Training module for Laboratory personne

Procedure

Inform the patient/guardian that there is a slight possibility of little scarring.

1. Explain the bleeding time procedure to the patients using words they can understand and obtain consent.
2. Record all medication used by the patient during the previous 7-10 days including over-the- counter items.
3. Wash or sanitize hands before touching the patient.
4. Position the patient sitting or lying down with the arm on a steady support and the volar surface exposed. (Palm of hand facing up).
5. Select a site on the muscular area of the forearm approximately 5cm below and parallel to the antecubital crease. If the patient is very hairy, shave the area lightly.
6. Remove the device from the blister pack, being careful not to contaminate or touch the blade slot surface.
7. Remove the safety clip but do not push the trigger yet.
8. Hold the device securely between the thumb and middle finger. Gently rest the device on the chosen site (parallel to the antecubital crease) and apply minimal pressure so that both ends of the device are lightly touching the skin.
9. Gently push the trigger, starting the stopwatch simultaneously.
10. Remove the device from the patient's forearm immediately after triggering and discard into the biohazard sharps container.
11. At 30 second intervals, wick (blot) the flow of blood from the wound by bringing the filter or blotting paper close to the incision. Do not touch the edges of the wound or disturb the platelet plug. The test is complete when the blood ceases to stain the filter paper.
12. The cut off time is 15 minutes. Record to the nearest 30 seconds.
13. Remove the cuff, clean the arm without disturbing the platelet plug. If bleeding continues for longer than 15 minutes, apply pressure to the wound. To reduce the potential for scarring, approximate the skin edges with a non-allergic wound closure such as Steri-strip and cover with a plaster. Advise the patient to keep the incisions covered for 24 hours.

Figure 50: Bleeding Time Procedure
Source: https://images.app.goo.gl/ShpFTT5mAqHVJX- 9RA

Bleeding time reference ranges:

1. Surgicutt Adults 2 – 8 minutes
2. Surgicutt jr 1.3 – 9 minute

References: https://diagnolab.com.na/images/ SOPs/SOP--306-BLEEDING-TIME.pdf

Coagulation Pathways

Coagulation is the formation of a blood clot and is essential to hemostasis. The coagulation process is characterized by a cascade of events which lead to the formation of a blood clot. Proteins called clotting factors initiate reactions which activate more clotting factors. This process occurs via two pathways which unite downstream to form the common pathway.

These are:

1. The extrinsic pathway: This is triggered by external trauma which causes blood to escape the circulation.
2. The intrinsic pathway: This is triggered by internal damage to the vessel wall.

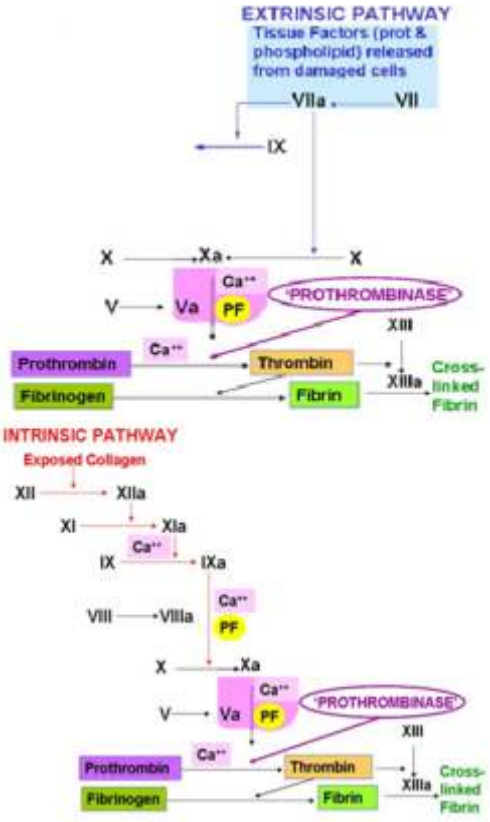


Figure 51: Blood Clotting Pathways
Source: https://images.app.goo.gl/VshZa2bYAWqveyxHA

Clotting time (CT)

The time takes for whole blood to clot is clotting time. It measures all stages of intrinsic coagulation. It is used to diagnose coagulation disorders or time taken for blood sample to clot in vitro. It is a less sensitive method.

Two methods are commonly used for Clotting time.

1. Capillary method
2. Tube method (Lee and white method)
3. Heparing retarded blood coagulation method

Capillary Method

Principal- Puncture the skin, blood is taken to a plain capillary tube and stopwatch started. Formation of fibrin strings is noted by breaking the capillary tube at regular intervals. Time taken for the first appearance of the fibrin string is noted.

Requirement: Disposable lancet, capillary tubing 10-15 cm long and 1.5 mm diameter

Procedure:

1. Warm up the finger for skin puncture
2. Make an incision with a sterile disposable lancet to a depth of 3 mm.
3. As soon as blood is visible start the stopwatch.
4. Wipe of the first drop of blood
5. Allow a second drop of blood to flow to the capillary tubing.
6. After 2 minutes break of the capillary tubing 1-2 cm from the end
7. When the thin string of fibrin can be seen in between the broken end of the capillary tube.
8. Stop the stopwatch and note the time.
9. Report the time.

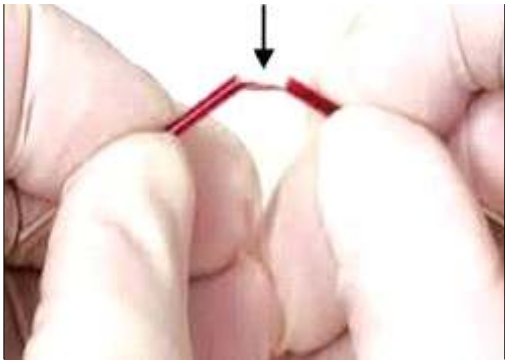


Figure 52: Capillary Method (Clotting Time Detection)
Source: https://images.app.goo.gl/MGu2JxVLgwTruJK4A

Box 1: Significance

- It appears to be the most valuable single testof coagulation.
- The test was used to detect hypercoagulableand hypo coagulable states.
- Prolonged clotting time seen in deficiency states involving anti-human globulin (AHG), plasma thromboplastin.
- Also prolonged in patients with bone marrow depression and thrombocytopenia

Normal Range CT: 1-5 min

Disadvantage:

1. Insensitive and unreliable method
2. Tube Method (Lee and White method)
3. Capillary blood always contaminates with tissue fluid.

Advantages

1. Can be performed when venous blood cannot be obtained.
2. No special equipment required.
3. Simple technique

Tube Method (Lee and White method)

Requirements: Glass tubes, water bath with thermometers, phlebotomy items

Procedure

1. Two siliconized tubes with a 10 cms external bore are taken.
2. These tubes are prewarmed at 37 °C in a water bath.
3. Take the blood sample, mostly taken from the antecubital vein.
4. 2 to 2.5 mL of blood is taken, and 1 mL of the blood is in each test tube.
5. Start two stopwatches as you see the blood in the syringe.
6. Keep the blood in the water bath and check for clotting by tilting each tube at 30 to 60 second intervals.
7. Tilt the tube to greater than 90 degrees.
8. Stop the stopwatch as you see the clot in the tube.
9. Clotting time is expressed as the mean of the two stopwatches.
10. Heparin retarded blood coagulation method

Disadvantage

1. This test is insensitive, so it lost its value.
2. There are many variables in the technique of performing the test.
3. This fails to detect the moderate deficiency of coagulation factors.
4. This test is only prolonged in severe deficiency.
5. Normal clotting time is despite prolonged bleeding time seen in thrombocytopenia.
6. This may be normal in patients taking anticoagulant therapy.
7. This is usually normal when the intrinsic and common pathways are present in an amount not exceeding 1% of the normal plasma level.
8. Because of all the above reasons, this test has lost its significance.

Heparin Retorted coagulation method

Heparin is a natural anticoagulant released from mast cells. It is used to decrease the clotting ability of the blood and help prevent harmful clots from forming in blood vessels.

This method is used to deep vein thrombosis or blood clot formation due to deficiency of heparin.

Requirement

1. 0.004 mg/L Heaparin solution
2. Venous blood freshly drawn into syringe.
3. Water bath with thermometer

Procedure

1. Add 1 ml heparin solution in a clen dry test tube in water bath at 37C.
2. Add 1ml of blood to this test tube and invert twice.
3. At the end of 12 min, tilt the tube gently at 1 min interval.
4. Look for clot formation.
5. Normal range 20-35 min

PROTHROMBIN TIME (PT)

- The PT test is used to monitor patients taking certain medications as well as to help diagnose clotting disorders.
- It involves the addition of brain thromboplastin to plasma, with clot formation after the addition of calcium chloride.
- Two laboratory tests are used commonly to evaluate coagulation disorders:
- Prothrombin Time (PT) which measures the integrity of the extrinsic system as well as factors common to both systems and Partial Thromboplastin Time (PTT), which measures the integrity of the intrinsic system and the common components.

Requirements

1. **Patient's Plasma:** Blood should be collected in 31.3g/l sodium citrate in a concentration of 1 volume citrate to 9 volume blood. As soon as after collection, the specimen should be centrifuged at about 3000 rpm for 10 min and the platelets poor plasma separated into a plastic tube using pasture pipette. This sample must be analyzed within 4 hours after collection, unless it is frozen and kept in the deep freezer until teste later.
2. **Normal Control plasma**: freshly collected plasma obtained in the same way as that of the patient.
3. **Rabbit brain Thromboplastin:** Stored at 4^0c Commercially obtained.
4. **Calcium Chloride Solution:** 0.025 mol/l, stored at 4^0c: in some commercial thromboplastin this has already been added.

Procedure

1. Reconstitute a vial of thromboplastin as per manufacturers instruction, keep it at water bath 37^0c for 10 min.
2. Dispense 0.1 ml into a plastic tube an add 0.1 ml of pre-warmed calcium chloride (or dispense 0.2 ml if thromboplastin-calcium I already combined)
3. Add 0.1 ml of pre-warmed plasma and start the stopwatch.
4. Tilt the tube gently every other second, keeping it as much as possible under water to maintain the temperature. Record the appearance of a fibrin clot at the end point and note time. The is called PT.
5. Perform the test on the patient plasma in duplicate, and also in duplicate on the normal control plasma. Repeat the test if duplicate measurements differ by more than 5%.

Normal range: It should be 13-17 seconds. However, this depends on the thromboplastin and should be established by testing a group of healthy subjects wherever a new reagent is introduced.

Reference: SEA/HLM/320, guidelines on standard operating procedures on Hematology, WHO

ACTIVATED PROTHROMBIN TIME (APTT)

- The activated partial thromboplastin time (aPTT) is a test performed to investigate bleeding disorders and to monitor patients taking an anticlotting drug such as heparin which inhibits factors X and thrombin, while activating anti- thrombin. This measures the intrinsic factor of coagulation pathway.
- aPTT measures the integrity of the intrinsic system (Factors XII, XI, VIII, IX) and common clotting pathways.
- Increased levels in a person with a bleeding disorder indicate a clotting factor may be missing or defective.
- At this point, further investigation is needed and warrants the use of sensitive assays for specific coagulation factors. Liver disease decreases production of factors, increasing the PTT.

Requirements

1. **Patient's Plasma**: Blood should be collected in 31.3g/l sodium citrate in a concentration of 1 volume citrate to 9 volume blood. As soon as after collection, the specimen should be centrifuged at about 3000 rpm for 10 min and the platelets poor plasma separated into a plastic tube using pasture pipette. This sample must be analyzed within 4 hours after collection, unless it is frozen and kept in the deep freezer until tested later.
2. **Normal Control plasma:** Freshly collected plasma obtained in the same way as that of the patient.
3. **Kaolin 5g/l in barbitone buffered saline pH 7.4**: This is stable at room temperature but should be stored at 4C.
4. **Phospholipid:** Available commercially, stored at -20C, it is stable for at least one year. It should be dispensed in small volumes and should not be frozen after throwing.
5. **Calcium Chloride:** 0.025 mol/l (i. e. M/40), stored at 4C

Procedure

1. Mix equal volume of phospholipid reagent and Kaoline suspension. Warmup in a glass tube in water bath at 37C
2. Place 01. ml of plasma in other glass tube. Add 0.2 ml of Kaolin-phospholipid reagent and start the stopwatch. Leave at 37 C for 10 min with occasional shaking.
3. At exactly 10 min add, 0.1 ml of prewarmed calcium chloride and start a second stop-watch.
4. Record the time taken for mixture to clot.
5. Perform the test on patient's plasma in duplicate, and also in duplicate in normal control plasma. Repeat the test if duplicate measurement differs by more than 5%.

Normal range: should be in the range of 30-40 sec, but this depends on the reagents used and preliminary incubation period. Each laboratory should establish its own normal range by testing a group of health controls.

THROMBIN TIME (TT)

This measures the time taken for plasma to clot when thrombin is added, it is a function of final phase of coagulation.

Requirements

1. **Patient's Plasma:** Blood should be collected in 31.3g/l sodium citrate in a concentration of 1 volume

citrate to 9 volume blood. As soon as after collection, the specimen should be centrifuged at about 3000 rpm for 10 min and the platelets poor plasma separated into a plastic tube using pasture pipette. This sample must be analyzed within 4 hours after collection, unless it is frozen and kept in the deep freezer until teste later.

2. **Normal Control plasma**: freshly collected plasma obtained in the same way as that of the patient.
3. **Barbitone buffered saline, pH 7.4**
4. **Thrombin**: This is available commercially as a freeze-dried material. It must be reconstituted with saline to 100 NH units per ml, dispensed in 1 ml aliquots and stored at -20C. for use a vial is thawed and diluted with barbitone-buffered saline, pH 7.4 to obtain a normal clotting time of about 17 second – usually about 7-8 unit/ ml.

Procedure

1. Add 0.1 ml of buffered saline to 0.1 ml of normal plasma and keep in water bath at 37C for 4 minutes.
2. Add 0.1 ml of thrombin and mix by shaking and simultaneously start the stopwatch.
3. Measure the clotting time.
4. Repeat with patient's plasma in duplicate followed by a second sample of the normal plasma.
5. Express results as a mean value for the patient and normal. Repeat the test with duplicate measurements differ by more than 5%.

Note: A patient's thrombin time should be within two seconds of the control. Times of 20 seconds and over are abnormal.

Interpretation of coagulation screening test

PT	APTT	TT	Possible Condition
N	N	N	Normal homeostasis, Thrombocytopenia (disorders of platelet function), vascular disorder, bleeding from severely damaged vessels, Very mild factor 8 (VIII) deficiency
Long	N	N	Factor 7 (VII) deficiency- rare, at start of oral anticoagulation therapy
N	long	N	Factor 8, 9 deficiencies, factor 11, 12 deficiencies (rare).
Long	Long	N	Vit K deficiency, oral anticoagulant drug, factor 2, 5, 10 deficiencies (rare)
Long	Long	Long	Heparin, fibrinogen deficiency, Hyperfibrinolysis

Reference: SEA/HLM/320, guidelines on standard operating procedures on Hematology, WHO

FIBRINOGEN DEGRADATION PRODUCT (FDP)

Fibrinogen (or fibrin) degradation products (FDPs) are fragments released following plasmin-mediated degradation of fibrinogen or fibrin. The d-dimer is a specific fragment formed only upon degradation of cross-linked fibrin.

Activation of the coagulation cascade results in thrombin generation, which in turn cleaves fibrinogen to form fibrin monomers. Cross-linked fibrin is the endpoint of the coagulation cascade Plasmin (as part of the fibrinolytic pathway) degrades fibrin (and fibrinogen), resulting in FDPs. One of the specific FDPs is fragment D. Dimers of this fragment (d-dimer) are detected only upon

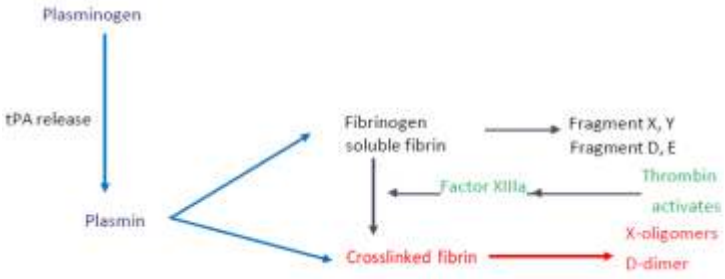


Figure 53: Fibrin Degradation Pathway
Source: https://images.app.goo.gl/4rCMDC9Eb- 8m3Z7DF7

degradation of cross-linked fibrin (indicating active coagulation and fibrinolysis).

FDP is tested by two methods.

1. Latex Agglutination
2. Enzyme immunoassays

Latex Agglutination

1. In Latex-based assays, particles are coated with antibodies to purified FDP fragments - usually Fragments D and E.

2. The suspension is mixed with a dilution of the serum sample to be tested and aggregation will occur if FDPs are present.

3. By using differing dilutions of the serum sample, a semi-quantitative assay for FDPs can be performed.

4. Serum FDP assays that use polyclonal antibodies will cross-react with Fibrinogen and this must be removed prior to testing.

5. This can be achieved either by the addition of Thrombin or by the use of specialized collection tubes containing Batroxobin [also known as Reptilase] - a snake venom isolated from the snake Bothropsatrox and inhibitors of Fibrinolysis.

6. Latex-agglutination assays utilizing monoclonal antibodies: In this assay, the use of monoclonal antibodies ensures that antibody does not cross-react with Fibrinogen and so the test can be used on citrated plasma samples.

Figure 54: Latex Agglutination Of Fdp Kit Component
Source: https://images.app.goo.gl/5kZNdwVfEHVanJt76

7. Latex Agglutination test may be set up as point of care diagnostic tool.

Enzyme Immunoassay/ ELISA

1. A microtiter plate is pre-coated with a Biotin-conjugated antibody specific to FDPs.
2. Standards or plasma samples are added to the appropriate wells and incubated.
3. Avidin conjugated Horseradish Peroxidase [HRP] is added to each well and incubated.
4. An HRP substrate [TMB] is then added and only those wells that contain FDPs, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color.
5. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at 450nm. The concentration of FDPs in the samples is then determined by comparing the OD of the samples to a standard curve.
6. TMB [3,3', 5,5”-Tetramethylbenzidine] is used as a substrate in a number of different assays. The reaction between the TMB substrate and a peroxidase, typically Horseradish Peroxidase [HRP], produces a measurable colour change that correlates with the analyte under investigation - in this case FDPs.
7. Follow the manufacturer's instructions given in the kit insert.

D DIMER

- The D-dimer test plays a very important role in diagnosis and monitoring of thrombosis and other diseases impacting blood coagulation.
- Primarily, it has an overriding importance in the exclusion of venous thromboembolism (VTE), in particular deep vein thrombosis and pulmonary embolism.
- Fibrinogen, the main protein of the blood coagulation system, becomes activated into Fibrin by Thrombin and Fibrin polymerization during the process of blood coagulation.
- Plasmin then digests the Fibrin clot and Fibrin degradation products of different molecular weights are released into the bloodstream.
- D-Dimer is the main and smallest product of Fibrin degradation, comprising of 111-197 amino acids in the α chain, 134-461 amino acids in the β chain, and 88-406 amino acids in the γ chain of Fibrinogen.
- All chains are cross-linked by disulfide bonds and the dimeric structure is held by two isopeptide bonds between C-terminal parts of γ chains.
- D-Dimer fragments can be measured easily in plasma and whole blood, and the presence or absence of D-Dimer may be useful in the diagnostic evaluation of venous thromboembolism.

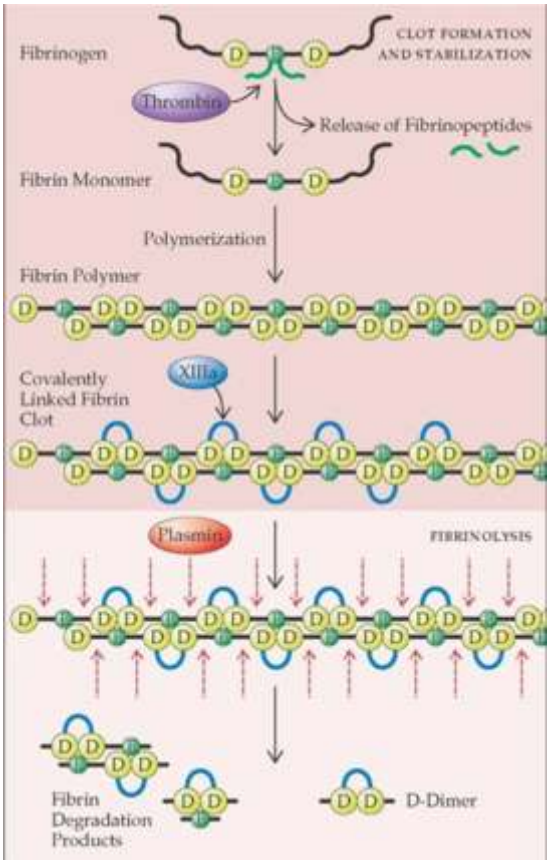


Figure 55: Clot Degradation Pathway
Source: https://images.app.goo.gl/52Z4KUae27TJhRhk9

Reference: Journal of Medical Sciences, 2007, Vol. 5, No. 1

Procedure: two methods are widely used for D dimer testing

1. ELISA testing
2. Agglutination testing

D- Dimer ELISA

- Quantitative ELISA assays are considered colloquially to be the reference standard for D-dimer quantitation.
- This method involves loading plasma specimens into microtiter wells coated with antibodies that have high affinity binding for D-dimers. After incubation, a labeled antibody is then added, and the quantity of bound, labeled substance is measured via colorimetric reaction.
- The labor-intensive and time-consuming constraints typical of conventional ELISA assays make them impractical for routine clinical laboratory use, which has spurred the development of more rapid, automated, and highly sensitive modified ELISA assays.
- Follow the manufacturer's instructions given in the kit insert.

D Dimer latex Agglutination

- Follow the manufacturer's instructions for testing procedure and kit storage condition

TEST FOR SICKLE CELL DISEASE

Sickle Cell Disease (SSD)

- Sickle cell disease (SCD) is a group of inherited red blood cell disorders.
- Red blood cells contain hemoglobin, a protein that carries oxygen.
- In someone who has SCD, hemoglobin is abnormal, which causes the red blood cells to become hard and sticky and look like a C-shaped farm tool called a "sickle.
- The sickle cells die early, which causes a constant shortage of red blood cells.
- Also, when they travel through small blood vessels, they get stuck and clog the blood flow.

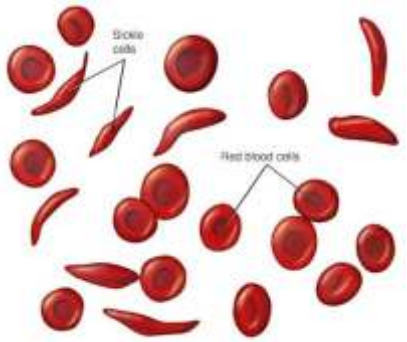


Figure 56: Sickle Cell And Normal Cell
Source: https://images.app.goo.gl/fBHbTkSR1SQqeJ3D6

- This can cause pain and other serious complications (health problems) such as infection, acute chest syndrome, stroke, Anemia, Venus thromboembolism and Kidney diseases.
- Sickle cell disease is genetic and transmitted in offspring in a Recessive Autosomal manner thus it is called Recessive Autosomal Trait (RAT).

Types of sickle cell disease

- **HbSS:** People who have this form of SCD inherit two genes, one from each parent, that code for hemoglobin "S." Hemoglobin S is an abnormal form of hemoglobin that causes the red cells to become rigid, and sickle shaped. This is commonly called sickle cell anemia and is usually the most severe form of the disease.
- **HbSC:** People who have this form of SCD inherit a hemoglobin "S" gene from one parent and a gene for a different type of abnormal hemoglobin called "C" from the other parent. This is usually a milder form of SCD.
- **HbS beta thalassemia:** People who have this form of SCD inherit a hemoglobin "S" gene from one parent and a gene for beta thalassemia, another type of hemoglobin abnormality, from the other parent. There are two types of beta thalassemia: "zero" (HbS beta0) and "plus" (HbS beta+). Those with HbS beta0-thalassemia usually have a severe form of SCD. People with HbS beta+-thalassemia tend to have a milder form of SCD.

Tests for Sickle cell disease:

1. Nestroft test for Osmotic fragility
2. DCIP test for E beta thalassemia screening
3. Solubility test for Hbs

NESTROFT TEST FOR OSMOTIC FRAGILITY

Principal: Normally, red cells put in saline solution begin to lyse at a saline concentration of 0.4-0.5% and lysis is complete at 0.32%. However, beta thalassemia trait, shows osmotic resistance due to alteration in volume/surface are of the affected RBC's.

Requirement: 0.36% buffered saline (BS) prepared by diluting 36ml of 1% buffered saline with 64ml of distilled water (DW) to make 100 ml (Test Reagent). Test tubes, Rular.

Procedure:

1. Two test tubes labelled as BS (2ml) and DW (2ml) taken.

2. Add drop of blood to each of the tubes
3. Left undisturbed for half an hour at room temperature.
4. The contents of both tubes were gently shaken and held against a white paper on which a thin black line was drawn.
5. The line was clearly the testable through DW tube and if it was the same in BS tube; it was considered negative, otherwise test result was interpreted as positive.

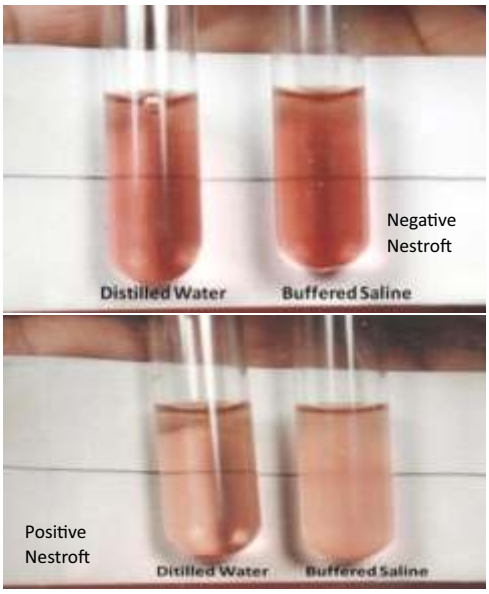


Figure 57: Nestroft Test For Osmotic Fragility
Source: https://images.app.goo.gl/j9KSKcCx38h-DZaFP9

DCIP test for E beta thalassemia screening

- In dichlorophenolindophenol precipitation (DCIP) test, 2,6-dichiorophenol indophenol (DCIP) were oxidizing chemicals and used as indicator of ascorbic acid measurement.
- Hemoglobin E is resulted from amino acid change at codon 26 of β-globin chain from glutamic acid to lysine.
- This change makes contact of α-globin chain and βE-globin chain less stable.
- Thus, in DCIP solution, molecule of HbE changes from tetramer to monomer, freeing sulfhydryl group of amino acid, oxidized by the DCIP, denatured, and precipitated.

Requirement:

- **DCIP reagent-** The 500-mL DCIP reagent is composed of 4.36 g Trizma base, 2.68 g EDTA- Na2.2H2O, 0.0276 g of DCIP, and 0.05 g of saponin. The pH of reagent is adjusted to 7.5 by using 6 N HCl. DCIP is available commercially.

Procedure

1. To perform the test, 20 µL of EDTA blood is mixed with 5 mL DCIP reagent.
2. The mixture is incubated at 37°C in water bath for 1 h before precipitation occurs in case of HbE carriers.
3. To enhance visualization, 20 µL of 6% (w/v) ascorbic acid is dropped into the mixture and the color of mixture turns from deep blue to pale red.

Normal percentage of 3 Hemoglobin

- Hb A- 95-98%
- HbA_2- 2 to 3%
- HbE- absent

SOLUBILITY TEST FOR HBS

Principal: This hemoglobin S screening test is based on the relative insolubility of hemoglobin S when combined with sodium dithionite, a reducing agent. When whole blood is mixed with the reducing agent, saponin lyses the erythrocytes and hemoglobin is released. If hemoglobin S is present, it will form liquid crystals and give a turbid appearance to the solution. A transparent solution is seen with other hemoglobin that are more soluble in the reducing agent.

Requirement

- **Stock:** Dissolve 216 gm K2HPO4 and 169 gm KH2PO4 in 1 lite of DW. Add 10 gm saponin in this reagent. Store at 4C (can used till 1 month).

- **Working:** Prepare sufficient quantity for the day by adding 5mg sodium dithionite (Na2S2O4) to 1mL of stock solution
- **Materials:** Glass test tubes, micropipettes (200 micro litre)', tips, 2 ml pipette, Paper-board test tube holder — reading card should have 14-point or 18-point black type in straight lines on a white background, approximately 0.5 cm apart.
- **Quality Control:** A positive control (A/S) containing 30-45% Hb S and a negative control (A/A) should be analyzed with each patient specimen.
- **Specimen:** Whole blood anticoagulated with EDTA, heparin, or sodium citrate is acceptable. Specimens

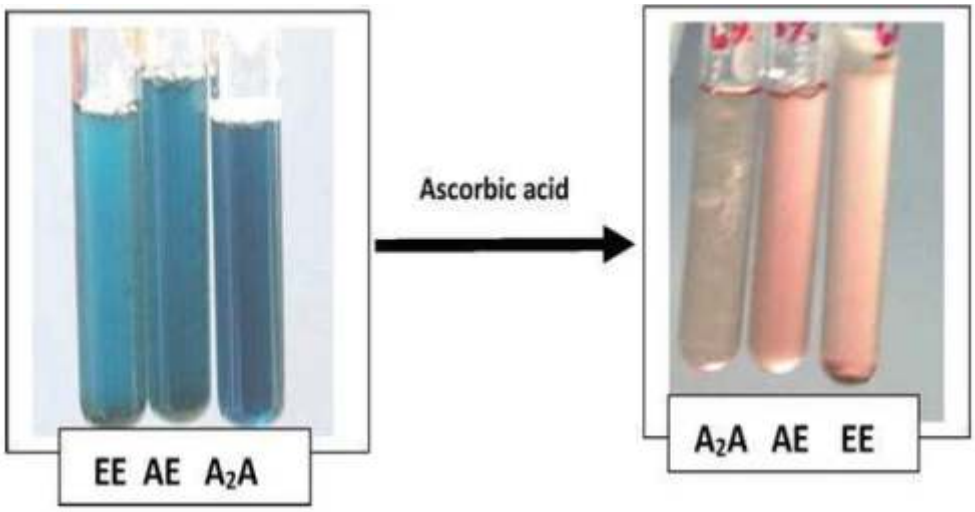


Figure 58: Dcip Test For E Beta Thalassemia (Ee- E Beta Thalassemia, Ae- E Beta Trait, A2a- Normal Hb)
Source: https://images.app.goo.gl/7wzdYwiFnt5LpGNk9

may be stored at 4C for up to three weeks before testing.

Procedure

Allow reagents and specimens to warm to room temperature prior to performing this test.

1. Pipet 2.0 mL of working solution into a labeled 12 x 75 mm test tube.
2. Add 0.02 mL of whole blood to the appropriately labeled test tube.
3. Mix the contents thoroughly.
4. Incubate the tubes for five minutes in the test tube holder at room temperature.
5. Read for turbidity.

Result: A positive result is indicated by a turbid suspension through which the ruled lines are not visible. A negative result is indicated by a transparent suspension through which the ruled lines are visible.

Figure 59: Solubility Test For Hbs
Source: https://images.app.goo.gl/yMRJG6mAkSwC- BEJm8

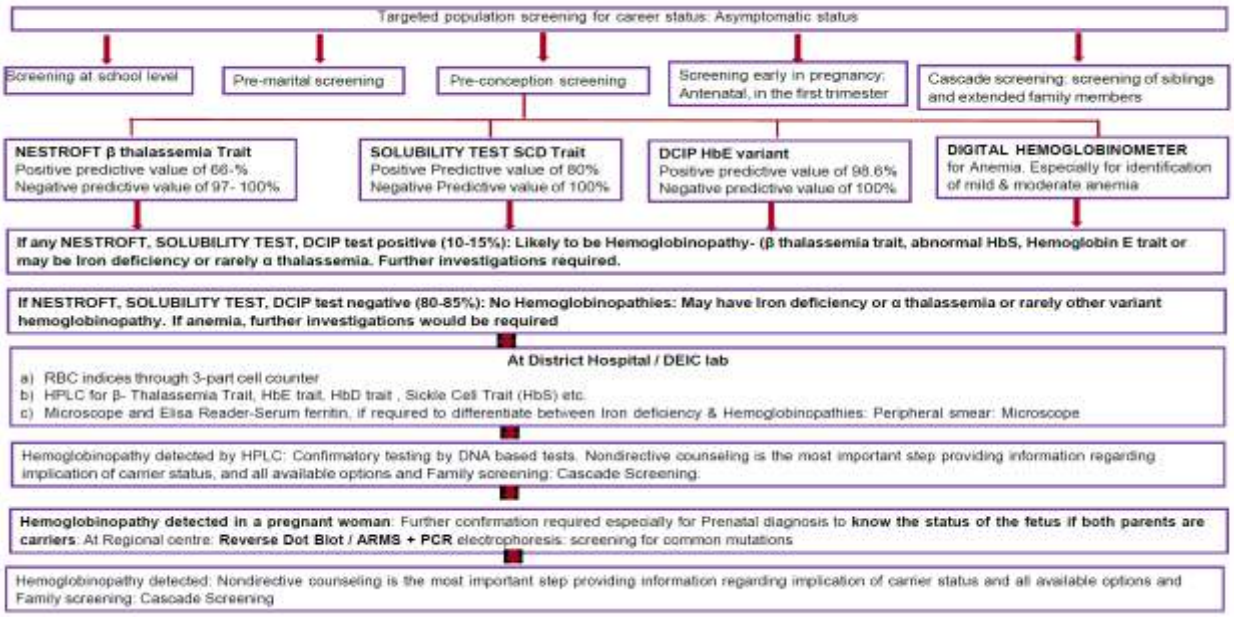


Figure 14: Algorithm For Population Screening for Hemoglobinopathies, Guidelines for Prevention of Hemoglobinopathies, MoHFW, GOI.

Source: Guidelines for Prevention of Hemoglobinopathies, MoHFW, GoI.

Reference

1. Guidelines for Prevention of Hemoglobinopathies, Ministry of Health and family welfare, GOI https://www.cdc.gov/ncbddd/ sicklecell/facts.html
2. https://www.jcdr.net/article_fulltext.asp?id=3759#:~:text=Two%20test%20tubes%20labelled%20as,thin%20black%20line%20was%20drawn.
3. J Clin Pathol 2006; 59:74–76. doi: 10.1136/jcp.2005.027961 wps.prenhall.com/wps/media/objects/684/700987/ch07SO.pdf
4. https://ashpublications.org/blood/article/4/1/66/6816/A-RAPID-DIAGNOSTIC- TEST-FOR-SICKLE-CELL-ANEMIA

Rapid diagnostic tests (RDT)

Rapid tests which are performed by either card/ strip/ agglutination are listed below. These tests are used in screening at point of care test (POCT).

General precautions for rapid test

- All reagents and samples should be at room temperature before testing.
- Follow appropriate sample collection procedure and required sample for test.
- Store reagents/ Kit at recommended temperature

S No.	Test name
1	Human chorionic gonadotropin (HCG) (Urine test for pregnancy)
2	Strip method of urine analysis
3	HIV (Antibodies to HIV 1&2)
4	Hepatitis B surface antigen test
5	Test for Filariasis- rapid
6	VDRL/ test for Syphilis
7	SARS CoV-2(COVID-19) Antigen
8	Malaria rapid test
9	HCV Antibody Test (Anti HCV) (Rapid)
10	Test for Dengue (Rapid)
11	Sickle cell test rapid for screening of Sickle cell anemia*(Strip test)
12	Screening test for G6PD enzyme defi-ciency
13	Typhoid test (IgM/IgG)
14	Typhoid testing methods (widal test quantitative and semiquantitative, Rapidtest of typhoid testing)
15	rK39 for Kala Azar*
16	Troponin - I/Troponin – T
17	G6PD test

- Do not use reagent/ kits beyond expiration date.
- Do not read rapid diagnostics test results after recommended time (may lead to false positive)
- Whole blood sample must be tested within 4-6 days of collection, if required storage, plasma/ serum may be separated.
- Follow kit insert and for detailed procedure and material safety data sheet (MSDS) of reagents and kits.

HUMAN CHORIONIC GONADOTROPIN (HCG) (URINE TEST FOR PREGNANCY)

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the placenta. Measuring hCG levels can be helpful in identifying a normal pregnancy. Urine testing is more convenient, affordable, comfortable for patients, has a fast turnaround (5 to 10 minutes), and does not require a medical prescription. It is the most commercially available and medical point of care test. An indicator (typically a colored line or symbol), along with a control, will appear if the test is positive. An isolated control line/symbol will be evident if the test is negative.

Procedure: Follow instructions given on the kit.

STRIP METHOD OF URINE ANALYSIS

- The Urinalysis Reagent Strips (Urine) are firm plastic strips onto which several separate reagent areas are affixed.
- The test is for the detection of one or more of the following analytes in urine: Ascorbic acid, Glucose, Bilirubin, Ketone (Acetoacetic acid), Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite and Leukocytes.
- Urinalysis is a useful procedure as an indicator of health or disease, and as such, is a part of routine health screening.
- The Urinalysis Reagent Strips (Urine) can be used in general evaluation of health, and aids in the diagnosis and monitoring of metabolic or systemic diseases that affect kidney function, endocrine disorders and diseases or disorders of the urinary tract.

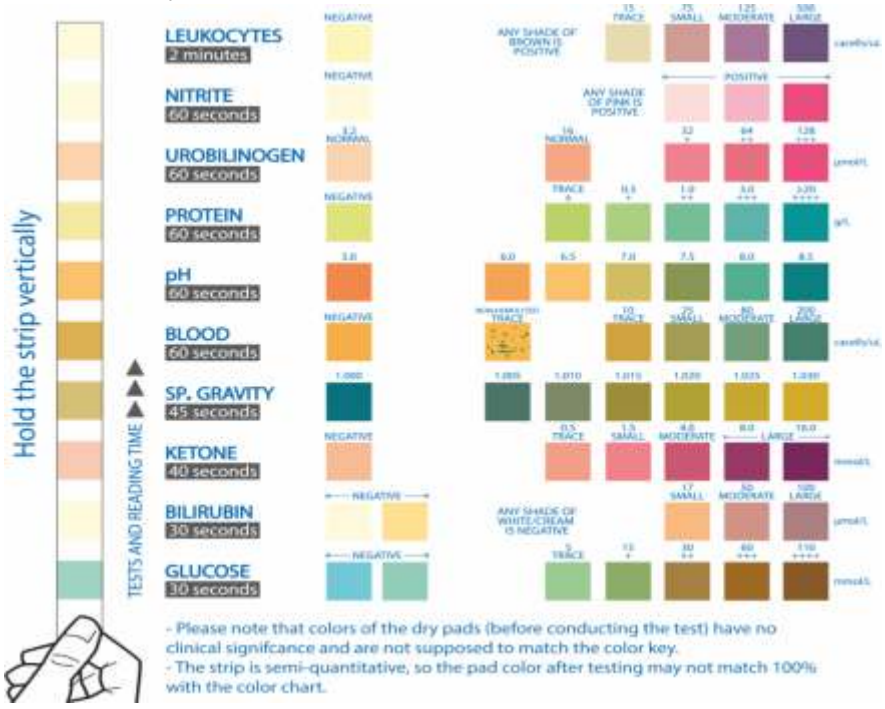


Figure 60: Strip Test For Urine
Source: https://images.app.goo.gl/rXyUrJ9dFf9HbS3e6

Specimen collection for Urine analysis and procedure

- A urine specimen must be collected in a clean and dry container and test as soon as possible. **Do not centrifuge.**
- If testing cannot be done within an hour after voiding, refrigerate the specimen immediately and let it return to room temperature before testing.
- Prolonged storage of unpreserved urine at room temperature may result in microbial proliferation with resultant changes in pH.
- A shift to alkaline pH may cause false positive results with the protein test area. Urine containing glucose may decrease in pH as organisms metabolize the glucose.
- Contamination of the urine specimen with skin cleansers containing chlorhexidine may affect protein (and to a lesser extent, specific gravity, and bilirubin) test results.
- Allow the strip, urine specimen, and/or controls to reach room temperature (15-30ºC) prior to testing.
- Follow the instructions given on kit.

Result Interpretation (Urine analysis)

- Results are obtained by direct comparison of the color blocks printed on the canister label.
- The color blocks represent nominal values; actual values will vary close to the nominal values.
- In the event of unexpected or questionable results, the following steps are recommended; confirm that the specimens have been tested within the expiration date printed on the canister label, compare results with known positive and negative controls and repeat the test using a new strip.
- Follow kit insert for interference of chemicals/ reagents.

VDRL/ SYPHILIS RAPID TEST

Syphilis is a curable infection caused by a bacterium called Treponema pallidum. This infection is sexually transmitted and can also be passed on from a mother to her fetus during pregnancy. As a cause of

genital ulcer disease, syphilis has been associated with an increased risk of HIV transmission and acquisition. Test is available on cassette and strip.

Procedure: follow instructions given in the kit insert.

SARS COV-2(COVID-19)

Antigen

- Antigen tests are immunoassays that detect the presence of a specific viral antigen, which indicates current viral infection.
- Antigen tests are currently authorized to be performed on nasopharyngeal, nasal swab, or saliva specimens placed directly into the assay's extraction buffer or reagent.
- The currently authorized antigen tests include point-of-care, laboratory-based, and self-tests.
- Antigen tests for SARS-CoV-2 are generally less sensitive than real-time reverse transcription polymerase chain reaction (RT-PCR) and other nucleic acid amplification tests (NAATs), which detect and amplify the presence of viral nucleic acid.
- However, NAATs may remain positive for weeks to months after initial infection and can detect levels of viral nucleic acid even when virus cannot be cultured, suggesting that the presence of viral nucleic acid may not always indicate contagiousness.
- Negative viral test results suggest no current evidence of infection. These results represent a snapshot of

the time around specimen collection and could change if the same test was performed again in one or more days.

- Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.
- **Procedure**: follow instructions given in the kit insert.

HCV ANTIBODY TEST (ANTI HCV) (RAPID)

Hepatitis C virus (HCV) causes both acute and chronic infection. Acute HCV infections are usually asymptomatic, and most do not lead to a life- threatening disease.

The HCV card test is rapid Immunochromatographic assay for the qualitative detection of antibodies to Hepatitis C Virus (HCV) in Human Whole Blood / Serum / Plasma. This test is a screening test, and all positives must be confirmed using an alternate test such as Western Blot.

HCV infection is diagnosed in 2 steps.

1. Testing for anti-HCV antibodies with a serological test identifies people who have been infected with the virus.
2. If the test is positive for anti-HCV antibodies, a nucleic acid test for HCV ribonucleic acid (RNA) is needed to confirm chronic infection and the need for treatment. This test is important because about 30% of people infected with HCV spontaneously clear the infection by a strong immune response without the need for treatment.

Procedure: follow instructions given in the kit insert.

SICKLE CELL RAPID SCREENING

- Rapid tool is a multiplexed, qualitative, point-of- care immunoassay to aid in the rapid diagnosis of sickle cell disorders.
- The test is made up of three indicators which detect the presence of hemoglobin A, S, and C, allowing the user to rapidly distinguish between normal, carrier, and sickle cell disease samples.
- Test kit provides results in 5 minutes, making it one of the fastest and most accurate tests in the world.

Figure 61: Sickle Cell Rapid Test (Example)
Source: https://images.app.goo.gl/kaDXtYyjhjfk2yET8

Test Procedure

- Collect a 5 µL blood sample.
- Mix blood with buffer solution.
- Place 5 drops on the test strip and wait 5 minutes.
- Follow directions given in kit insert.

GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD)

Glucose 6 phosphate dehydrogenase is the enzyme found in cell cytoplasm. This enzyme is crucial for maintaining red blood cell healthy by generating glutathione during the pentose phosphate pathway which protects erythrocytes from oxidizing agents (reactive oxygen species).

- If levels of G6PD are too low, hemoglobin will not bind oxygen and the red blood cell wall will break, resulting in hemolysis. G6PD deficiency is the most common inherited sex- linked enzyme deficiency, that affects more than 400 million people worldwide, and mostly populations throughout Africa, Asia, The Mediterranean and the Middle East.

- Deficiency is inherited on the X chromosome and since males have just one X chromosome, they can be hemizygous G6PD normal or hemizygous G6PD deficient.

G6PD genotypes and phenotypes

Correlation of G6PD and P. Vivax infection

- P. vivax is the most widespread plasmodium species and was responsible for 13.8 million cases of malaria in 2015.
- P. vivax produces both a blood stage and a liver stage of infection.
- In liver parasites remain as dormant hypnozoites which can be reactivated to result in recurrent clinical attacks, unless hynozoites are specifically targeted with 14 days treatment using the 8- aminoquinoline drug, primaquine.
- While primaquine results in radical cure of P. vivax, use in individuals with G6PD deficiency can induce severe haemolysis which may result in death.

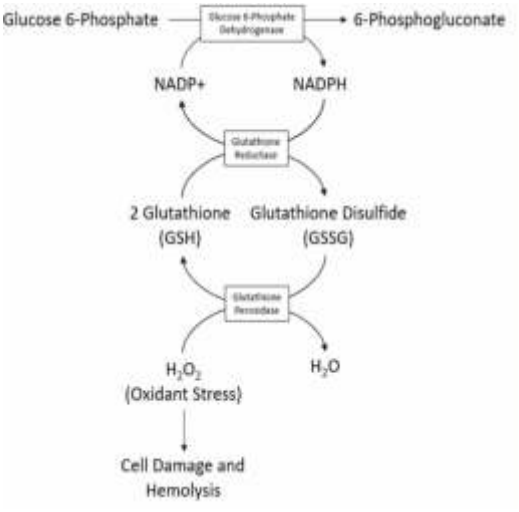


Figure 62: G6PD deficiency
Source: Https://Www.ncbi.nlm.nih.gov/Books/ Nbk470315/

- The risk of inducing haemolysis while treating P. vivax infected patients with primaquine therefore presents a serious public health issue and a potential roadblock for elimination.
- Consequently, G6PD testing is recommended prior to administration of primaquine and forms a crucial component of P. vivax control and elimination programs. Laboratory testing of G6PD activity need to do before prescribing primaquine as per the WHO guidance.
- **G6PD test RDT method**: follow the instructions given in kit insert.

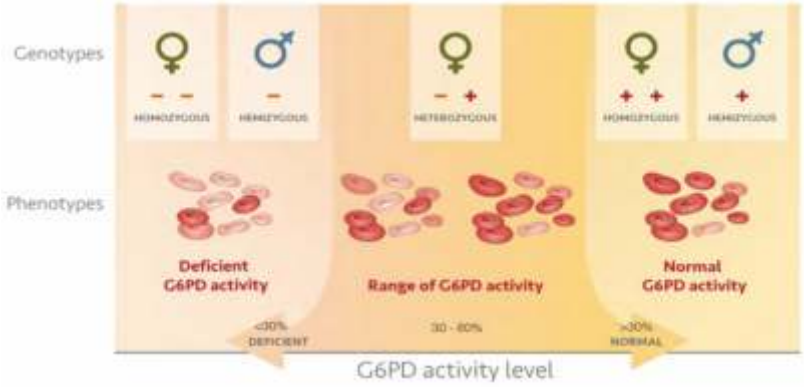


Figure 63: Source: WHO Report, Guide to G6PD Deficiency Rapid Diagnostic Testing To Support P. Vivax Radical Cure,
Https://Www.who.int/Publications/I/Item/9789241514286
Source: https://images.app.goo.gl/1bLEhsqNvqRfeB6H6

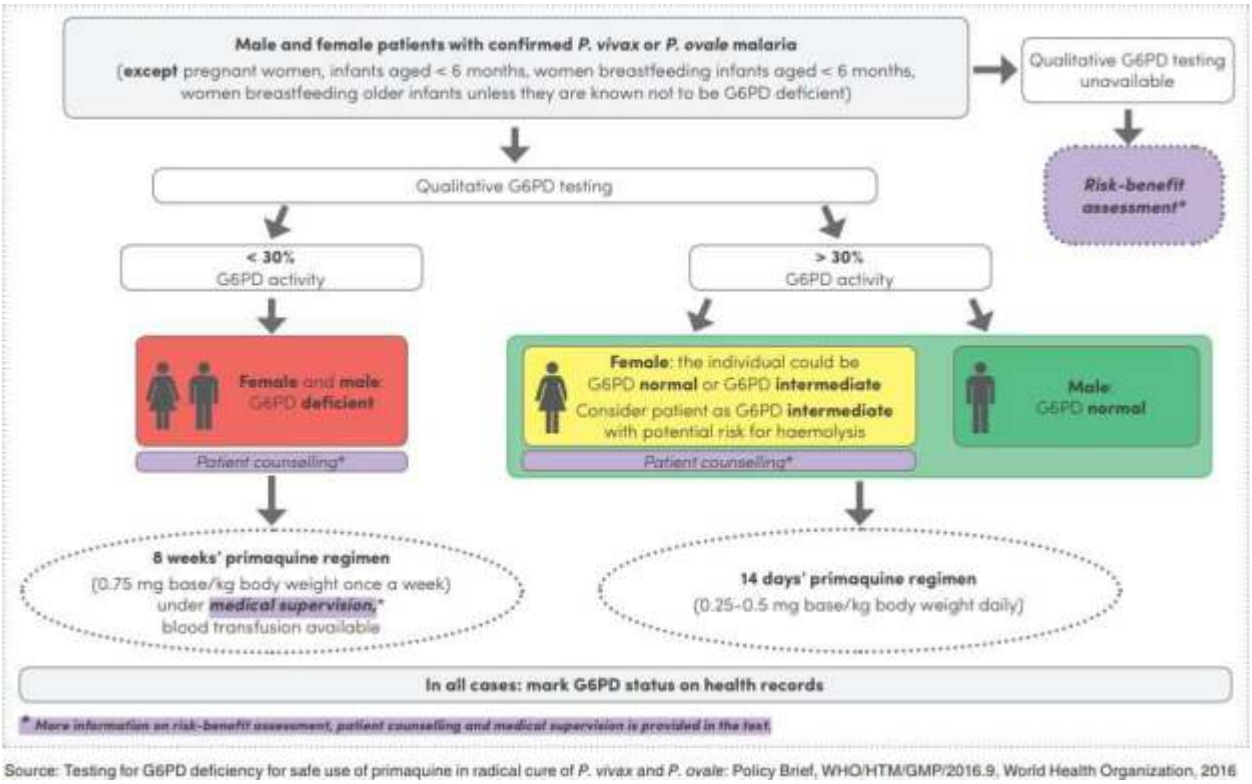
TYPHOID TEST (IGM/IGG)

TYPHIDOT Rapid IgG/IgM (Combo) is an immunochromatographic assay designed for the qualitative detection and differentiation of specific IgM and IgG antibodies against specific Salmonella typhi OMP antigen in human serum or plasma. It is intended to be used as an in vitro diagnostic of typhoid fever.

Principle: Rapid IgG/IgM (Combo) is an indirect solid phase immunochromatographic assay. The specific S. typhi OMP antigen is immobilized onto cellulose nitrate membrane as test lines. When the test sample is added to the sample pad, it migrates upwards. If anti-S. typhi IgG and IgM antibodies are present in the test sample (serum or plasma), they will react with colloidal gold-anti-human IgG or gold- anti-human IgM to

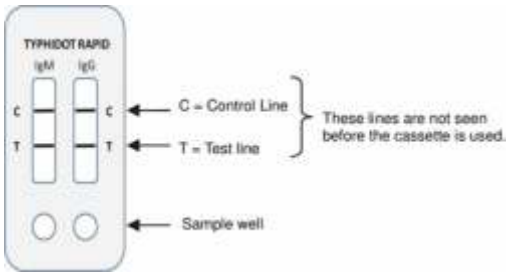
form a complex. The complex will continue to move on the cellulose nitrate membrane and then captured at the test window zone by the immobilized specific S. typhi OMP antigen, giving a pink purplish colored band. The control line contains rabbit anti-mouse IgG antibody which binds with the gold conjugated mouse anti-human IgG or mouse antihuman IgM antibodies. The control band serves as an indication of proper migration plus reagent control.

Procedure: follow instructions given in the kit insert.



Source: Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale*: Policy Brief, WHO/HTM/GMP/2016.9, World Health Organization, 2016

Figure 64: Recommendation (Https://Www.who.int/Publications/I/Item/9789241514286)
Source: https://images.app.goo.gl/owh1gxyHgFhNz9Q39



Source: https://www.reszonics.com/wp-content/up - loads/2017/04/Reszon-IFU-TYPHIDOT-Rapid-IgG_IgM-combo-2014-04.pdf

KALA AZAR

- Leishmaniasis is caused by a protozoa parasite from over 20 Leishmania species. Over 90 sandfly species are known to transmit Leishmania parasites.
- Visceral leishmaniasis (VL), also known as kala-azar, is fatal if left untreated in over 95% of cases.
- Cutaneous leishmaniasis (CL) is the most common form and causes skin lesions, mainly ulcers, on exposed parts of the body.

- Mucocutaneous leishmaniasis leads to partial or total destruction of mucous membranes of the nose, mouth and throat.

rK39 test for Kala Azar (rapid)

- rK39 is a protein containing 39 amino acid repeats derived from a conserved region within a gene coding for a kinesin-related protein of L. infantum.
- Visceral Leishmaniasis (VL) is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to members of L. donovani in human serum to aid in the presumptive diagnosis of VL.
- Required sample- Serum (not whole blood)
- Tests should be performed as soon as possible after sera collection.
- Bring sera to room temperature prior to testing. The frozen sera must be completely thawed prior to testing. Sera should not be repeatedly frozen and thawed.

Procedure: follow instructions given in the kit insert.

TROPONIN - I/TROPONIN – T

- Cardiac troponin I (cTnl) is a protein found in cardiac muscle.
- Troponin I is part of 3 subunit complex comprising of troponin T and troponin C along with tropomyosin. This structure complex forms the main component that regulates the calcium sensitive ATPase activity of actinomycin in striated skeletal and cardiac muscle.
- After occurring cardiac injury, troponin I released into blood 4-6 hours after onset of pain. The release pattern of cTnl is similar to CK-MB, but while CKMB levels return to normal after 72 hrs. Troponin I remails elevated for 6-10days, thus providing a longer window of detection for cardiac injury.
- Troponin I has become the most preferred biomarker for Myocardial infarction.

Procedure: follow instructions given in the kit insert.

CHAPTER - 18

BIOCHEMISTRY EQUIPMENTS

BIOCHEMISTRY ANALYZER

Purpose: To describe the procedure for the operation and maintenance of the Biochemistry Analyzer

Scope: This SOP describes how to use and maintain a Biochemistry Analyzer. This performs automated counts and requires no manual operations for aspirating blood, dilutions, measuring, calculations, printouts, and computer transfer used in the Biochemistry laboratory.

Responsibility:

It is the responsibility of Lab – In-charge of the lab to train lab technician/assistant and students on this procedure and to ensure adherence to this procedure. It is the responsibility of the students/ technicians to follow the SOP as described and to inform the Lab In-Charge about any deviations or problems that may occur while performing the procedure.

Standard Operating Procedure For Biochemistry Analyzer

Procedure:

1. Check the Analyzer is attached with Electric supply.
1. Switch on the Analyzer
2. Press the Aspiration key to take water, wash it thrice.
3. Keep the blank and press the aspiration button.
4. Keep the standard solution and press the aspiration bottom.
5. Keep the test sample and press the aspiration button and record the reading.
6. Wash using water.
7. Switch the Analyzer

Precautions To Be Followed

- Keep away from direct Sunlight.
- Keep in a dry and cool space.

Record To Be Maintained

- Laboratory Manual containing the experiments that can be performed with the equipment.
- Maintenance Record

Calibration

1. Frequency of calibration varies between tests and depends on the workflow.
2. Calibration is required when an existing calibration expires, when reagents are replaced and when control results fall outside specified acceptable ranges refer to (Interpretation of QC data).

Samples Collection and storage

1. Collect blood samples according to the blood sample collection and handling SOPs.
2. Stability during storage varies between serum parameters. If analyses are not performed on the day of collection, store serum samples at minus 20°C.
3. Volume required: > 500µl.

4. Exclusion criteria: severe hemolysis.

Sample preparation:

1. Prepare the serum samples collected on the same day of the measurement by centrifuge them at ~5000 x g for 3 -5 minutes.
2. If necessary, remove fibrinogen clots using a wooden applicator.
3. Use serum samples undiluted or diluted to a ratio of 1:2 with deionized water or normal saline if the volume is insufficient.
4. Load the racks according to the work lists.

Quality Control:

1. Each morning, all parameters are tested with control sera. Some parameters are tested with control serum level 1 and control serum level 2, which consists of lyophilised human plasma with a normal and a pathological concentration. Other parameters are tested with specific controls from other suppliers.
2. Controls are thawed and vortexed before utilization and loaded according to the analyzer's display. Control values must lie within the acceptable range indicated by the manufacturer, otherwise the specific tests must be recalibrated, and specific measurements repeated. Controls can be stored in 200µl aliquots at -20°C for up to 1 week.

Analyzing results:

1. Samples that produce results that lie outside the linear range for a specific assay have to be re-tested. In some cases, it may be necessary to dilute samples with water to bring test results into range.
2. Validate the data.
3. Transfer the data to the database.

Laboratory Errors

Analytical errors are classified into random errors and systematic errors. Random errors indicate poor precision while systematic errors indicate poor accuracy. A few examples of random errors are pipetting error, transcription error, wrong sample numbering and labeling, and fluctuating readings on the colorimeter.

Systematic errors could occur due to wrong procedure, incorrect standard, and calibration procedure. Errors can occur in any of the limbs of the cycle of events taking place in a hospital, starting from the physician examining the patient and back to the physician (preanalytical/ analytical/ post analytical).

Construction of Levey Jennings Chart

On each day when analyses are performed a fresh sample is thawed, thoroughly mixed and analyzed. (Remember: Ethanediol-treated serum may not freeze completely at – 20^0C; however, the constituents are quite stable). The QC serum is analyzed for a period of 20 days or so. [Important to note: Analysis should not be carried out by only one person; all staff should participate in this exercise to determine the true unavoidable error in the laboratory]. From these data, mean and SD (standard deviation) are calculated. Levey Jennings chart is then constructed with x + 2SD as warning limits and x + 3 SD as control limits.

Calculate the %CV (coefficient of variation) for each analyte to ascertain whether this is within the acceptable limit (Ideal = < 5%. Must be definitely < 8%). If % CV is found to be high, this will indicate that between-day laboratory precision (variation) is high and the data cannot be used to construct a Levey Jennings chart. It is then essential to identify the causes for this, correct these and then repeat the whole exercise and confirm that the %CV is well within the acceptable limit.

Interpretation of QC data

A. According to WHO an analytical system is 'out of control' if one of the four criteria is met. That is:

- a value lies entirely outside the control limits.
- seven consecutive values show a rising tendency.
- seven consecutive values show a falling tendency.

- seven consecutive values lie on the same side of the mean.

If one of these situations arises, the patients' results must be discarded, the cause of the error sought and removed, and then the batch repeated with a QC serum.

B. Use of two different levels of QC simultaneously in every batch of analysis provided valuable information on the type of errors – whether these are random (precision) errors.

or

systematic (accuracy) errors.

Maintenance of Biochemistry Analyzer

There is a lot to consider in maintaining a Chemistry Analyzer as small dust particles, dirt, oil, or fingerprints within the system can lead to inaccurate results. Here's some elements of the machines that need cleaning or regular attention.

Cleaning the Electronic Boards and Internal Components

Step 1: Dismantle the cover of the machine. It will help us access the electronic board, exhaust fan, and other parts inside the machine.

Step 2: Clean the electronic circuit boards using the Chemtronics Duster and Chemtronics dry wipes to ensure 100% cleanliness and eliminate dirt or dust particles. Do not reuse wipes as they can cross-contaminate or spread soil into more sensitive components.

Cleaning the Optical System

Step 1: Dismantle the Optical system and the halogen lamp.

Step 2: Remove the dirt or dust particles on the lamp's surface using Optic Prep Pre-Saturated Wipes. Avoid touching the lamp's surface to prevent contamination via fingerprint, and don't re-use wipes as soil can scratch or contaminate.

Step 3: Use cleanroom swabs and Optic Prep Pre- Saturated Wipes to clean the surfaces of the lens to remove dirt or dust particles. Don't re-use swabs or wipes on these sensitive surfaces.

Step 4: Use the Chemtronics Aerosol Duster to obtain ultra-pure cleanliness of the lenses.

Step 5: Clean the overall body of the Optical system using Chemtronics dry wipes for final touches.

Step 6: Reinstall the Optical System, lamp, and return the cover.

Cleaning the Cuvette

Step 1: Remove the cuvettes from the reaction disk or the flow cell.

Step 2: Place the cuvette onto an Optic Prep Pre-Saturated Wipe and clean its surface using Optic Prep Pre-Saturated Wipes and Coventry™ cleanroom swabs. These will remove excess oils or existing fingerprints. Don't touch the cuvette surface; fingerprints and human-generated oils are some of the reasons for dirty cuvettes. Do not reuse swabs and wipes which could redeposit soil.

Step 3: Clean the reaction disk or the flowcell using Coventry™ cleanroom swabs, then reinstall the cuvettes in the system.

Overall External Cleaning

To ensure 100% cleanliness, we need to clean the overall machine. Using the Coventry cleanroom wipes & Chemtronics dry wipes, we can ensure 100% cleanliness and a dirt or dust-free workstation or laboratory.

What if you are the next patient to be tested and the laboratory machines are not well-maintained? Do you want to receive an incorrect diagnosis? That's why machine maintenance is a must but using the correct maintenance products is a plus.

References

https://www.researchgate.net/

1. publication/277709909_Standard_Operating_Procedures_For_Clinical_Chemistry

2. Guidelines on Standard Operating Procedures for Clinical Chemistry, WHO, SEA-HLM-328, https://apps.who.int/iris/rest/bitstreams/911202/ retrieve

URINE ANALYZER

- Follow instrument manual provided.
- Company engineers must provide training to all staff for instrument operation.
- What is the purpose of Urine analyzer?
- Reading test urine strip semi automatically (strip to dip in urine and placed in analyzer). To overcome the manual reading errors.
- Potential errors of manual strip reading: improper lighting at the workplace, or different color discrimination by the user or different timing when the values are read.
- The urine test strips that shall be used with the instrument are multi parameter strips for the determination of specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, ascorbic acid and blood in urine.
- The Urine Analyzer is a reflectance photometer. The strip is illuminated by white light, and the reflected light from the strip is detected by the Sensor. The RGB signals are digitized, and this digitized image is interpreted by the processor. The intelligent image analyzer SW locates the strip and the pads, and based on these color data the parameter values are determined.

The results including the date and time of the measurement, sequence number and the ID are stored and printed out by the internal printer.

Urine analyzer- Caution

- Never use bend strip
- Remove excess urine on the strip by using soft tissue paper.
- Clean instrument daily to prevent malfunction (If the machine is carefully kept free of excess urine then daily cleaning may not be necessary)
- The instrument calibrates manually.
- Wear personal protective equipment.
- Use universal precautions.
- Do not wipe the body clean with benzene, thinner, gasoline, etc

Urine Aanalyzer: Calibration

- The Calibration of the instrument should be done prior to first use and then the same process.
- From the 2nd time can be recommended to do in every 4 weeks with Calibration Strip in the package.
- Calibration Strip is used for checking aging processes of the optical system and variation of other internal conditions of instrument.
- When strong variation is detected which may be caused, for instance, by contamination of the reference pad or low light intensity of a defective light source, an error message will be printer
- The Calibration Strips are white plastic standard strips with defined and constant reflectance characteristics.
- The Calibration Strip should remain in the vial until use and should be used only once.
- Do not touch the middle areas of the strip.
- The plate strip tray should be clean and dry before a calibration is performed.

Urine Analyzer: Quality Control

- Performance of reader result should be confirmed by testing known negative and positive specimen or controls whenever a new bottle is first opened or calibration.
- Water should NOT be used as a negative control.

- Controls should be tested after performing maintenance or service on the instrument.
- Quality Control materials should be used in accordance with local, state, and/or federal guidelines.

ELECTROLYTE ANALYZER

- Electrolyte analyzer is intended to be used for the measurement of sodium, potassium, chloride, ionized calcium, and lithium in samples of whole blood, serum, plasma, urine, dialysate and aquas standard solution.
- Principal: methodology is based on ion selective electrode. Analyzer may have 6 electrodes (sodium, potassium, chloride, ionized calcium, lithium and a reference electrode).
- Each electrode has an ion selective membrane that undergoes a specific reaction with corresponding ion contained in the sample being analyzed.
- Membrane is an ion exchanger reacting to the electrical charge.
- Follow instrument manual for operation, maintenance, and calibration.

CHAPTER - 19

BIO MEDICAL WASTE MANAGEMENT (BMWM)

Waste Management

- Municipal Solid Waste
- Dry waste to be discarded in blue bags - Paper, plastic, bottles etc.
- Wet Waste to be discarded in green bags – Food waste etc.
- Biomedical Waste (described later)

► Segregation is the responsibility of the person generating the waste.
► All lab personnel must be trained and comply with requirements of BMWM rules
► BMW Management: safe disposal of BMW is a part of the hospital waste management

► **Benefits:**

- Protects Health & Environment
- Reduces Cost of health care
- Ensures Biosafety

► **Guidelines:**

- Institutional/Local/Regional/ National
- Latest guidelines BMW rules 2016, amended 2018

Bio Medical Waste Management (BMWM)

S. No.	Colour coding	Type of container	Type of waste	Final treatment / disposal
1	Yellow	Autoclave safe plastic bags or containers	Soiled waste – items contaminated with blood, body fluids Microbiology & biotechnology waste; Non sharps non plastic solid waste	Incineration (certain items may require pre-treatment before handing over to CBMWTF)
2	Red	Non chlorinated plastic bags or containers	Contaminated recyclable plastic wastes	Autoclaving / microwaving (certain items may require pre-treatment before handing over to CBMWTF)
3	White	Puncture proof, leak proof, tamper proof containers	Waste metallic sharps	Decontamination at CBMWFT followed by recycling
4	**Blue**	Puncture proof, leak proof, tamper proof containers	Waste glass items	Decontamination at CBMWFT followed by recycling

CHAPTER - 20

INVENTORY MANAGEMENT, QMS (IQC AND EQA) AND CERTIFICATION

Inventory Management

Inventory management in a laboratory setting is crucial to ensure smooth operations, maintain adequate supplies, and support timely and accurate testing processes. Here are some key considerations for inventory management in a lab:

1. **Inventory categorization**: Categorize your inventory based on the type of materials, chemicals, reagents, consumables, or equipment required for lab operations. This helps in organizing and tracking inventory effectively.
2. **Minimum stock levels:** Determine the minimum stock levels for each inventory category. Set thresholds to trigger reordering when the stock reaches a predefined quantity to avoid stockouts and disruptions in lab activities.
3. **Regular stock monitoring**: Conduct regular stock checks to assess the availability and condition of inventory items. Keep track of expiration dates for chemicals and reagents to prevent using outdated materials that could compromise test results.
4. **Usage tracking:** Keep records of inventory usage to analyze consumption patterns and anticipate future requirements accurately. This data can assist in optimizing procurement decisions and avoiding unnecessary overstocking.
5. **Vendor management:** Maintain relationships with reliable suppliers to ensure timely delivery of inventory items. Establish clear communication channels and negotiate favorable terms to streamline the procurement process.
6. **Equipment maintenance:** Track and schedule maintenance for lab equipment to prevent unexpected breakdowns and ensure uninterrupted testing processes. Maintain a record of equipment service history and calibrations.
7. **Digital inventory management systems**: Implement a laboratory information management system (LIMS) or specialized inventory management software to automate inventory tracking, facilitate reordering, and generate reports for analysis and decision- making.
8. **Safety considerations:** Pay attention to safety protocols for hazardous materials or chemicals in the lab. Store and handle them properly, following appropriate regulations and guidelines to ensure the well-being of lab personnel and the integrity of the inventory.

By effectively managing inventory in a lab, you can optimize resources, minimize waste, and maintain the necessary supplies to support efficient and accurate laboratory operations.

Points to Remember

- Maintain records.
- Stock logbook should include name and signature, date of receipt, quantity, date of expiry, minimum stock, stock balance.
- Inspect new orders on delivery: verify contents, check integrity, record date each item received, record expiration date, store new shipment behind, existing shipment, create or update records.

- **Storeroom:** Should be clean, organized, lockable, well-ventilated, no direct sunlight, good, sturdy shelving, items within reach, safety precautions should be available
- **Shelf organization:** avoids "losing" a product, saves time, systemizes storage space.
- Opened reagent should be clearly labeled "Date of Open."
- Reagent, buffer and other reagents (like staining reagents, media, disinfectants etc.) prepared in lab should be marked as "date of preparation and date of expiry".

Quality Management System

What is a Quality Management System?

A quality management system (QMS) is a set of processes and procedures that ensure the quality of products or services. In the context of a laboratory, a QMS is used to ensure the quality of laboratory tests and results.

A QMS typically includes the following elements:

► **Management commitment:** The laboratory management must be committed to quality and must provide the resources necessary to implement and maintain a QMS.

There are some management commitments of QMS elements for public health laboratories:

à *Leadership:* The laboratory director or other senior manager must provide leadership and commitment to quality.

à *Policy:* The laboratory must have a written policy that outlines the laboratory's commitment to quality.

à *Resources:* The laboratory must provide the necessary resources to implement and maintain a QMS.

à *Communication:* The laboratory must communicate the QMS to all staff and ensure that they understand their roles and responsibilities in ensuring quality.

à *Review:* The laboratory must regularly review the QMS to ensure that it is effective and meets the needs of the laboratory.

► **Documentation:** The laboratory must have a documented QMS that includes policies, procedures, and work instructions. The documents should include:

à *Policies and procedures:* The laboratory should have a set of policies and procedures that outline the laboratory's quality management system (QMS). These policies and procedures should cover all aspects of laboratory testing, from sample collection to reporting of results.

à *Work instructions:* The laboratory should have work instructions for all of its laboratory tests. These work instructions should provide detailed instructions on how to perform the tests and how to interpret the results.

à *Records:* The laboratory should keep accurate records of all laboratory testing. These records should include the following:

- o The identity of the patient or specimen
- o The date and time of the test
- o The test results.
- o Any problems that occurred during the testing

à *Training records:* The laboratory should keep training records for all laboratory staff. These

records should show that all staff have been trained on the QMS and their roles and responsibilities in ensuring quality.

- **Training:** All laboratory staff must be trained on the QMS and their roles and responsibilities in ensuring quality. All laboratory staff should be trained on the QMS and their roles and responsibilities in ensuring quality. The training should cover the following topics:
  - *The laboratory's QMS:* The training should provide an overview of the laboratory's QMS, including its policies, procedures, and work instructions.
  - *The importance of quality:* The training should emphasize the importance of quality in laboratory testing and how it can impact patient care.
  - The role of laboratory staff: The training should explain the role of laboratory staff in ensuring quality, including their responsibilities for following procedures, reporting problems, and maintaining records.
  - *The importance of documentation:* The training should emphasize the importance of accurate and complete documentation in laboratory testing.
  - *The principles of quality control:* The training should cover the principles of quality control, including how to monitor the performance of tests and identify and correct problems.
- **Equipment and facilities:** The laboratory must have the appropriate equipment and facilities to perform the tests and to ensure the quality of the results.The equipment and facilities should be appropriate for the types of tests that are performed in the laboratory. They should also be properly maintained and calibrated the key considerations for equipment and facilities in public health laboratories under QMS:
  - *The type of tests that are performed:* The equipment and facilities in the laboratory should be appropriate for the types of tests that are performed. For example, a laboratory that performs molecular tests will need different equipment and facilities than a laboratory that performs routine microbiology tests.
  - The volume of testing: The volume of testing that is performed in the laboratory will also affect the type of equipment and facilities that are needed. For example, a laboratory that performs a high volume of testing will need more equipment and facilities than a laboratory that performs a low volume of testing.
  - The budget: The budget for the laboratory will also affect the type of equipment and facilities that can be purchased.
  - The regulatory requirements: The regulatory requirements for public health laboratories will also affect the type of equipment and facilities that are needed. For example, some regulatory bodies require that laboratories have specific types of equipment for certain types of testing.
- **Quality control (QC)**: The laboratory must have a system of quality control in place to monitor the performance of the tests and to identify and correct any problems. In a laboratory is a set of procedures and activities used to ensure the accuracy and reliability of laboratory test results. QC measures are designed to identify and correct errors in the laboratory process before they result in inaccurate results.

There are two main types of QC measures:

Internal QC: These measures are performed within the laboratory to monitor the performance of the laboratory process. Internal QC measures typically include the use of quality control materials, instrument calibration, and proficiency testing.

Establishing the value range for the control material

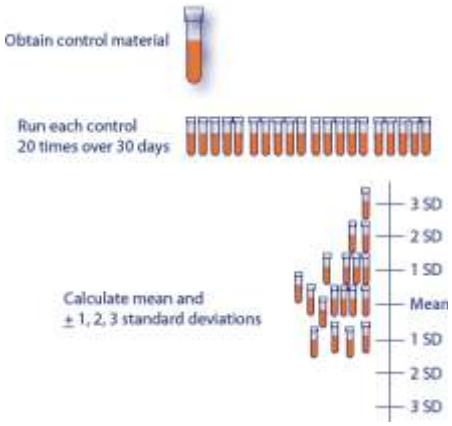


Figure 89: Who Lqms Manual (Ref)

Once the appropriate control materials are purchased or prepared, the next step is to determine the range of acceptable values for the control material. This will be used to let the laboratory know if the test run is "in control" or if the control values are not reading properly-"out of control". This is done by assaying the control material repeatedly over time. At least 20 data points must be collected over a 20–30-day period. When collecting this data, be sure to include any procedural variation that occurs in the daily runs; for example, if different testing personnel normally do the analysis, all of them should collect part of the data.

Once the data is collected, the laboratory will need to calculate the mean and standard deviation of the results. A characteristic of repeated measurements is that there is a degree of variation. Variation may be due to operator technique, environmental conditions, or the performance characteristics of an instrument. Some variation is normal, even when all of the factors listed above are controlled. The standard deviation gives a measure of the variation. This process is illustrated.

Graphically representing control ranges

Once the appropriate range of control values has been established, the laboratory will find it very useful to represent the range graphically for the purpose of daily monitoring. The common method for this graphing is the use of Levey–Jennings charts.

Run the control and plot it on the Levey–Jenning's chart.

- If the value is within +2 SD, the run can be accepted as "in-control".
- In this case, the second value is "out of control" because it falls outside of 2 SD.- Run must be rejected and explore the possible cause of errors.

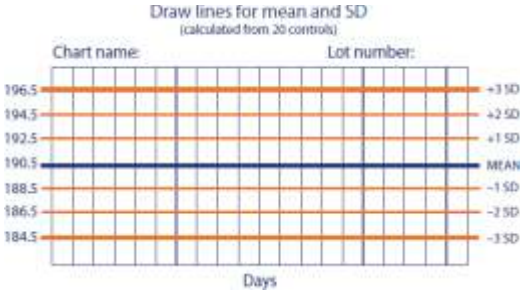


Figure 90: Who Lqms Manual (Ref)

Errors may be of two types- Random errors and Systematic errors. With random error, there will be a variation in QC results that show no pattern. This type of error generally does not reflect a failure in some part of the testing system and is therefore not like to reccur. Random error is only a cause for rejection of the test run if it exceeds +2 SD.

Systematic error is not acceptable, as it indicates some failure in the system that can and should be corrected. Examples of evidence of systematic error include:

- shift—when the control is on the same side of the mean for five consecutive runs.
- trend—when the control is moving in one direction and appears to be heading toward an out-of-control value.

Shifts in the mean occur when an abrupt change is followed by six or more consecutive QC results that fall on one side of the mean, but typically within 95% range as if clustered around a new mean. On the sixth occasion this is called a shift and results are rejected

Trends occur when values gradually, but continually, move in one direction over six or more analytical runs. Trends may display values across the mean, or they may occur only on one side of the mean. On the sixth occasion, this is determined to be a trend and results are rejected.

Using quality control information

When the QC sample that is used in a test run is out of the acceptable range, the run is considered to be "out of control". When this happens, there are several steps that the laboratory must follow.

à The testing process should be stopped and the technologist must immediately try to identify and correct problems.

à Once possible sources of error have been identified and corrections have been made, the control material should be rechecked. If they read correctly, then patient samples, along with another QC specimen, should be repeated. Do not simply repeat the testing without looking for sources of error and taking corrective action.

à Patient results must not be reported until the problem is resolved and the controls indicate proper performance.

When attempting to solve QC problems, it is useful to have established policies and procedures for remedial action. Often, manufacturers of either equipment or reagents will provide guidelines that can be helpful. Use any troubleshooting guides that are available.

Possible problems to consider include:

- à degradation of reagents or kits
- à control material degradation
- à operator error
- à failure to follow manufacturer's instructions.
- à an outdated procedure manual
- à equipment failure
- à calibration error.

External QC: These measures are performed by an outside agency to assess the overall quality of the laboratory. External QC measures typically include participation in proficiency testing programs and the accreditation of the laboratory by a recognized organization.

The benefits of implementing QC measures in a laboratory include:

- Increased accuracy and reliability of test results
- Reduced risk of errors and mistakes
- Improved efficiency and productivity
- Enhanced patient and treating physicians' satisfaction.
- Compliance with regulatory requirements

QC Measures used in the laboratory:

1. **Use of quality reagents:** Quality reagents are known reference materials that are used to assess the accuracy and precision of laboratory tests.

   The use of quality reagents in public health laboratories is essential to ensure the accuracy and reliability of laboratory tests. Reagents are chemicals and other substances used in laboratory tests to measure the concentration of an analyte. The quality of reagents can have a significant impact on the accuracy and reliability of laboratory tests. There are many different types of reagents available, and the quality of reagents can vary widely. Some reagents are of high quality and are manufactured to strict

standards. Other reagents may be of lower quality and may not be manufactured to the same standards. The use of low-quality reagents can lead to inaccurate and unreliable laboratory test results. This can have serious consequences for public health, as inaccurate test results can lead to misdiagnoses and incorrect treatment decisions.

For this reason, it is important to use high- quality reagents in public health laboratories. When selecting reagents, it is important to consider the following factors:

The quality of the reagent manufacturer*:* Some reagent manufacturers have a better reputation for quality than others. It is important to select a reagent manufacturer that has a good reputation for quality.

The quality of the reagent: The quality of the reagent can be assessed by reviewing the reagent's certificate of analysis. The certificate of analysis should list the reagent's composition, purity, and accuracy.

The expiry date of the reagent: Reagents have an expiry date, and it is important to use reagents before their expiry date. Using expired reagents can lead to inaccurate and unreliable laboratory test results.

2. **Instrument calibration:** Instruments must be calibrated regularly to ensure that they are providing accurate results.

Improved accuracy and reliability of test results: Calibration ensures that instruments are accurate, which means that the results of laboratory tests are also accurate.

Reduced risk of errors: Maintenance helps to keep instruments in good working order, which reduces the risk of errors during laboratory procedures.

Increased efficiency and productivity: Calibration and maintenance can help to identify and correct problems with instruments, which can lead to increased efficiency and productivity in the laboratory.

Compliance with regulatory requirements: Many regulatory bodies require that laboratories calibrate and maintain their instruments. By doing so, laboratories can demonstrate compliance with these requirements.

There are many different ways to calibrate and maintain instruments in public health laboratories. The most common methods include:

Using certified reference materials: Certified reference materials are known standards that are used to calibrate instruments.

Using in-house standards: In-house standards are prepared in the laboratory and used to calibrate instruments.

The specific method that is used will depend on the type of instrument and the availability of resources.

Calibration and maintenance of instruments is an essential part of laboratory quality assurance in public health laboratories. By following a regular schedule for calibration and maintenance, laboratories can ensure that their instruments are accurate and reliable, which will help to ensure the accuracy and reliability of laboratory test results.

Here are some of the key considerations for instrument calibration and maintenance in public health laboratories:

The type of instrument: The type of instrument will determine the frequency of calibration and maintenance. For example, analytical instruments may need to be calibrated more frequently than laboratory equipment.

The environment in which the instrument is used: The environment in which the instrument is used can also affect the frequency of calibration and maintenance. For example, instruments that are used in

a humid environment may need to be calibrated more frequently than instruments that are used in a dry environment.

The availability of resources: The availability of resources, such as certified reference materials and trained staff, will also affect the frequency of calibration and maintenance.

This plan should be reviewed and updated on a regular basis to ensure that it is still appropriate for the needs of the laboratory.

3. **Proficiency testing:** Proficiency testing is a program in which laboratories participate to assess their performance against other laboratories. Proficiency testing (PT) is an important tool for ensuring the quality of laboratory testing in public health laboratories. By participating in PT, public health laboratories can identify and correct any problems with their testing procedures. PT can also help to improve the accuracy and reliability of laboratory test results.

There are many different types of PT programs available for public health laboratories. Some of the most common PT programs include:

External PT programs: External PT programs are offered by commercial companies or organizations. These programs provide participating laboratories with samples that have been prepared by a central laboratory. The participating laboratories then analyze the samples and submit their results to the central laboratory. The central laboratory compares the results of the participating laboratories to a reference value and provides feedback to the laboratories on their performance.

Internal PT programs: Internal PT programs are developed and administered by individual public health laboratories. These programs use samples that have been prepared by the laboratory itself. The participating laboratories then analyze the samples and submit their results to the laboratory. The laboratory compares the results of the participating laboratories to a reference value and provides feedback to the laboratories on their performance.

The specific requirements of PT programs for public health laboratories will vary depending on the program. However, most PT programs include the following steps:

Sample preparation: The central laboratory or the public health laboratory prepares the PT samples. The samples are prepared in a way that is consistent with the way that samples are prepared in the participating laboratories.

Distribution of samples: The central laboratory or the public health laboratory distributes the PT samples to the participating laboratories. The samples are usually distributed in sealed containers to ensure that they are not tampered with. Analysis of samples: The participating laboratories analyze the PT samples using their own testing procedures.

Submission of results: The participating laboratories submit their results to the central laboratory or the public health laboratory. The results are usually submitted electronically.

Comparison of results: The central laboratory or the public health laboratory compares the results of the participating laboratories to a reference value. The reference value is the value that is expected for the PT samples.

Feedback to laboratories: The central laboratory or the public health laboratory provides feedback to the participating laboratories on their performance. The feedback may include information on the accuracy and reliability of the laboratories' results, as well as any areas where improvement is needed.

4. **Accreditation:** Accreditation is a process by which a laboratory is evaluated against a set of standards to ensure its quality.

There are a number of organizations that offer accreditation to public health laboratories in India. These include:

National Accreditation Board for Testing and Calibration Laboratories (NABL): NABL is the national accreditation body for testing and calibration laboratories in India. It is a non-profit organization that was established in 1987 by the Department of Science and Technology, Government of India. NABL's accreditation scheme is based on the international standard ISO/IEC 17025:2017.

Clinical Laboratory Accreditation (CLA): CLA is a private organization that offers accreditation to clinical laboratories in India. It was established in 2005 and its accreditation scheme is based on the international standard ISO 15189:2012.

Association of Public Health Laboratories (APL): APL is a non-profit organization that was established in 1974 by the Indian Council of Medical Research (ICMR). APL's accreditation scheme is based on the international standard ISO 17025:2017.

The accreditation process for public health laboratories in India typically involves the following steps:

1. The laboratory applies for accreditation to the chosen accreditation body.
2. The accreditation body conducts an initial assessment of the laboratory to assess its compliance with the relevant standards.
3. If the laboratory is found to be compliant, it is awarded provisional accreditation.
4. The laboratory is then required to undergo a periodic surveillance audit to ensure that it continues to meet the relevant standards.
5. If the laboratory continues to meet the relevant standards, it is awarded full accreditation.

Accreditation of public health laboratories in India is voluntary. However, many laboratories choose to be accredited in order to demonstrate their commitment to quality and to improve the confidence of their clients in the accuracy and reliability of their test results.

The specific QC measures that are used in a laboratory will depend on the type of laboratory and the tests that are performed. However, all laboratories should have a comprehensive QC program in place to ensure the quality of their test results.

The key principles of quality control in a laboratory:

Accuracy: The results of laboratory tests should be accurate, meaning that they should be close to the true value of the analyte being measured. This is important for public health laboratories because

they are often used to diagnose and track diseases, and inaccurate results could lead to incorrect decisions about treating patients and public health interventions.

Precision: The results of laboratory tests should be precise, meaning that they should be reproducible. This is important for public health laboratories because they often use standardized methods and reagents, and reproducible results ensure that the results of different laboratories are comparable.

Reliability: The results of laboratory tests should be reliable, meaning that they should be consistent over time. This is important for public health laboratories because they often use long-term monitoring programs, and reliable results ensure that the trends observed over time are accurate.

Timeliness: The results of laboratory tests should be timely, meaning that they should be available when they are needed. This is important for public health laboratories because they often provide results to support public health interventions in situations of pandemic and outbreak, and timely results ensure that the interventions are effective.

Safety: Laboratory tests should be conducted safely, to protect the health and safety of laboratory staff and the public. This is important for public health laboratories because they often handle hazardous materials, and

safe practices ensure that the laboratory staff and the public are not exposed to harm.

Compliance: Public health laboratories must comply with regulatory requirements, which often include specific standards for quality. This is important for public health laboratories because it ensures that the results of their tests are accurate, precise, reliable, timely, safe, and compliant with regulations.

By following these principles, laboratories can ensure that their test results are accurate, precise, reliable, and timely. This is essential for ensuring the quality of patient care and for meeting regulatory requirements.

- **Internal audits:** The laboratory must conduct regular internal audits to assess the effectiveness ofthe QMS. They help toensure that the laboratory is meeting its quality objectives and that the QMS is effective.

  The internal audit process should be documented and should include the following steps:

  1. Planning: The audit should be planned in advance and should include the following:
     - o The scope of the audit
     - o The objectives of the audit
     - o The audit criteria
     - o The audit schedule
  2. Auditing: The audit should be conducted by a qualified auditor who is independent of the area being audited. The auditor should collect evidence to determine whether the laboratory is meeting its quality objectives and that the QMS is effective.
  3. Reporting: The auditor should report the findings of the audit to the laboratory management. The report should include the following:
     - o The audit scope
     - o The audit objectives
     - o The audit criteria
     - o The audit findings
     - o The audit recommendations
  4. Follow-up: The laboratory management should take action to address the audit findings and recommendations.

- **External assessment:** The laboratory may also be required to undergo external assessment by a regulatory or accreditation body.

  External assessment in public health laboratories is the process of having an independent organization assess the laboratory's quality management system (QMS) to determine whether it meets the requirements of a particular standard, such as ISO 17025:2017.

  External assessment is an important part of the QMS in public health laboratories. It helps to ensure that the laboratory is meeting the requirements of the standard and that the QMS is effective.

  The external assessment process should be documented and should include the following steps:

  1. Planning: The assessment should be planned in advance and should include the following:
     - o The scope of the assessment
     - o The objectives of the assessment
     - o The assessment criteria

- The assessment schedule

2. **Assessment:** The assessment should be conducted by a qualified assessor who is independent of the laboratory being assessed. The assessor should collect evidence to determine whether the laboratory is meeting the requirements of the standard and that the QMS is effective.
3. **Reporting:** The assessor should report the findings of the assessment to the laboratory management. The report should include the following:

- The assessment scope
- The assessment objectives
- The assessment criteria
- The assessment findings
- The assessment recommendations

4. **Follow-up:** The laboratory management should take action to address the assessment findings and recommendations.

The benefits of implementing a QMS in a laboratory include:

- Increased accuracy and reliability of test results
- Reduced risk of errors and mistakes
- Improved efficiency and productivity
- Enhanced patient and treating physicians' satisfaction
- Compliance with regulatory requirements

There are many different standards that can be used to implement a QMS in a laboratory. Some of the most common standards include:

- ISO 15189:2012 Medical laboratories - Requirements for quality and competence
- CLSI GP26-A3 Laboratory Quality Management System
- CAP QMS Laboratory Quality Management System

The specific standard that is used will depend on the type of laboratory and the regulatory requirements that apply.

However, the benefits of doing so can be significant. By implementing a QMS, laboratories can improve the quality of their tests and results, reduce the risk of errors, and improve patient and treating physicians' satisfaction.

External Quality Assessment (EQA)

The term EQA is used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory. This comparison can be made to the performance of a reference NABL Certified laboratory.

Types of EQA

Several EQA methods or processes are commonly used. These include.

Proficiency testing: External provider sends unknown samples for testing to a set of laboratories, and the results of all laboratories are analyzed, compared and reported to the laboratories.

Rechecking or retesting: Slides that have been read are rechecked by a reference laboratory; samples that have been analyzed are retested, allowing for inter laboratory comparison.

On-site evaluation: Usually done when it is difficult to conduct traditional proficiency testing or to use the rechecking/retesting method.

EQA benefits

Participation in an EQA program provides valuable data and information, which:

- allows comparison of performance and results among different tests sites.
- provides early warning for systematic problems associated with kits or operations.
- provides objective evidence of testing quality.
- indicates areas that need improvement.
- identifies training needs.

Individual laboratories can use EQA to identify problems in laboratory practices, allowing for appropriate corrective action. EQA participation will help to evaluate reliability of methods, materials and equipment, and to evaluate and monitor training impact.

For laboratories performing public health–related testing, EQA can help to ensure that results from different laboratories during surveillance activities are comparable. EQA participation is usually required for accreditation. Also, EQA participation creates a network for communication, and can be a good tool for enhancing a national laboratory network. Samples received for EQA testing, as well as the information shared by the EQA provider, are useful for conducting continuing education activities.

Principal characteristics of an EQA scheme

Principal characteristics include the following:

- EQA programs requires a fee. (Free EQA programs include those offered by a manufacturer) to ensure equipment is working correctly, and those organized by a regional or national programme for quality improvement.
- Some EQA programs are obligatory or voluntary enrollment is done in order to achieve improvement in the quality of the laboratory's performance.
- The EQA program can be organized at different levels: regional, national or international.
- Individual laboratory results are kept confidential, and generally are only known by the participating laboratory and the EQA provider. A summary is generally provided and allows comparison to the overall group.

Proficiency testing

Proficiency testing is the most employed type of EQA, as it is able to address many laboratory methods.

PT is available for most of the commonly performed laboratory tests, and covers a range of chemistry, hematology, microbiology, and immunology testing.

Proficiency testing process

In the PT process, laboratories receive samples from a PT provider usually from NABL certified laboratory that serves as the reference organizations

The laboratories participating in the program analyze the samples and return their results to the central organization. Results are evaluated and analyzed, and the laboratories are provided with information about their performance and how they compared with other participants. The participating laboratories use the information regarding their performance to make appropriate changes and improvements.

Participation in EQA

All laboratories should participate in EQA challenges, and this should include EQA for all testing procedures

performed in the laboratory.

EQA Management Process

When participating in EQA programs, the laboratory needs to develop a process for the management

of the process. A primary objective is to assure that all EQA samples are treated in the same manner as other samples tested. Procedures should be developed that address:

- **Handling of samples**: These will need to be logged, processed properly and stored as needed for future use.
- **Analyses of samples:** Consider whether EQA samples can be tested so that staff do not recognize them as different from patient samples (blinded testing).
- **Appropriate record keeping**: Records of all EQA testing reporting should be maintained over a period of time, so that performance improvement can be measured.
- **Investigation of any deficiencies**: For any challenges where performance is not acceptable.
- **Taking corrective action when performance is not acceptable:** The purpose of EQA is to allow for detection of problems in the laboratory, and to therefore provide an opportunity for improvement.
- Communication of outcomes to all laboratory staff and to management

EQA performance problems

If the laboratory performs poorly on EQA, the problems may lie anywhere along the path of workflow. All aspects of the process will need to be checked. Some examples of problems that may be identified include the following.

Pre-examination:

- The sample may have been compromised during preparation, shipping, or after receipt in the laboratory by improper storage or handling.
- The sample may have been processed or labeled improperly in the laboratory.

Examination:

- The EQA challenge materials may exhibit a matrix effect in the examination system used by the participating laboratory.
- Possible sources of analytical problems include reagents, instruments, test methods, calibrations and calculations. Analytical problems should be investigated to determine whether error is random or systemic.
- The competence of staff will need to be considered and evaluated.

Post-examination:

- The report format can be confusing.
- Interpretation of results can be incorrect.
- Clerical or transcription errors can be sources of error.

Incorrect data captured by the EQA provider is another possible source of error, which should be taken care of.

Certification of Public Health Laboratories

Under the overall ambit of the Quality Assurance Programme of GoI, The Quality initiatives and accreditations shall be undertaken to define the mechanism of National Quality Assurance Standards (NQAS) and External Quality Assurance Scheme (EQAS) for Public Health Laboratories.

For the public health facility should be certified with NQAS and their laboratories should be accredited by NABL.

Nation Quality Assurance Standards (NQAS) is a framework developed by NHSRC to improve the quality of healthcare services across the country. It is a set of nationally recognized standards and guidelines that aim to enhance the quality of health services, ensure patient safety, and standardize healthcare practices in different healthcare facilities.

The NQAS covers various levels of healthcare facilities, including District hospitals, Community Health Centers, Primary Health Centers, HWCs etc. The standards are designed to be applicable at these different levels to ensure that each facility maintains a certain level of quality in service delivery.

For example, in the context of laboratories, NQAS would outline specific quality assurance and quality control measures to be followed. These measures would ensure accurate and reliable laboratory testing, proper handling of samples, adherence to safety protocols, and robust documentation practices.

The NQAS measurement system comprises different elements that work together to assess and monitor the quality of healthcare services in a facility. These elements are interrelated and together contribute to evaluating and improving the overall quality of care. The relationship between these elements can be summarized as follows:

1. **Standards and Guidelines:** The foundation of the NQAS measurement system is the set of established standards and guidelines. These standards outline the specific requirements and expectations for the quality of care in different areas of healthcare, such as clinical services, infrastructure, safety, and patient experience.
2. **Indicators and Metrics:** Indicators are measurable elements that help evaluate performance and adherence to established standards. Each standard may have one or more associated indicators or metrics. Indicators serve as quantifiable benchmarks to track progress and assess compliance with the NQAS.
3. **Data Collection and Reporting:** Data collection is a crucial part of the measurement system. Facilities must gather relevant data related to the defined indicators regularly. This data can come from various sources, including patient records, surveys, audits, and quality improvement initiatives. The collected data is then used for reporting purposes.
4. **Reporting and Feedback:** The collected data is analyzed, aggregated, and reported to the relevant stakeholders, including administrators, healthcare providers, and policymakers. This feedback loop provides an understanding of the facility's performance in relation to the NQAS standards and helps identify areas that require improvement.
5. **Assessment and Evaluation:** Based on the reported data, the facility undergoes assessments or evaluations to gauge its compliance with the NQAS standards. These evaluations can be internal, external, or even conducted by accrediting bodies.
6. **Quality Improvement Initiatives:** The measurement system highlights areas of concern and identifies opportunities for improvement. Based on the evaluation results, the facility can implement targeted quality improvement initiatives to enhance the quality of care and align with NQAS standards.
7. **Monitoring and Continuous Improvement:** The measurement system operates in a cyclical manner, with continuous monitoring of performance, regular assessments, and ongoing quality improvement efforts. It creates a culture of continuous learning and refinement of processes to maintain and elevate the quality of care.

Courtesy:https://qps.nhsrcindia.org/sites/default/ files/2022-04/Operational-Guidelines-for-Improving_ Quality_Public_Health_Facilities_2021.pdf

These are a set of standards and guidelines established by the government or relevant healthcare authorities to ensure the quality and efficiency of healthcare services, including those provided by laboratories.

Source: https://qps.nhsrcindia.org/sites/default/files/2022-04/Operational-Guidelines-for-Improving_Quality_Public_ Health_Facilities_2021.pdf

As a Laboratory Technician, your responsibilities related to NQAS would include:

1. **Compliance with NQAS Guidelines:** Familiarize yourself with the specific NQAS guidelines relevant to your laboratory's scope of work. Ensure that all laboratory procedures, processes, and practices adhere to these standards.
2. **Quality Control and Assurance:** Implement and participate in quality control and assurance programs as outlined by NQAS. This involves regular monitoring, evaluation, and documentation of laboratory processes and results to maintain and improve service quality.
3. **Accurate Documentation:** Keep detailed and accurate records of all laboratory activities, including sample handling, testing procedures, and results. This documentation is essential for complying with NQAS requirements and for potential audits.
4. **Proficiency Testing:** Participate in proficiency testing programs as required by NQAS to assess the accuracy and reliability of laboratory testing. This may involve testing samples with known values to ensure the laboratory's results are consistent and accurate.
5. **Equipment Maintenance:** Ensure that all laboratory equipment is properly maintained, calibrated, and functioning correctly. Regular maintenance is crucial to producing accurate and reliable results, which aligns with NQAS standards.
6. **Continuing Education and Training:** Stay updated with the latest developments in laboratory science and technology through ongoing education and training. This ensures that you and your colleagues are well-equipped to meet the changing requirements of NQAS and provide high-quality services.
7. **Participation in Audits:** Cooperate with internal and external audits conducted to assess the laboratory's compliance with NQAS guidelines. Address any identified deficiencies and implement corrective actions promptly.

8. **Risk Management:** Identify potential risks and hazards in laboratory processes and work to mitigate them. This may involve developing and implementing safety protocols to protect staff and patients and prevent errors.

9. **Participate in Quality Improvement Initiatives:** Actively engage in quality improvement initiatives within the laboratory. Collaborate with colleagues and management to identify areas for improvement and implement evidence-based strategies to enhance service quality.

10. **Communication:** Effectively communicate with laboratory staff, supervisors, and other healthcare professionals to ensure seamless coordination and understanding of NQAS requirements and their implementation.

NABL (National Accreditation Board for Testing and Calibration Laboratories):

- NABL is an autonomous body under the Department of Science and Technology, Government of India. It is the leading accreditation body for testing and calibration laboratories in India.
- NABL provides accreditation to laboratories based on the ISO/IEC 17025 standard. This standard specifies the general requirements for the competence of testing and calibration laboratories and covers areas such as quality management, personnel competency, equipment calibration, and traceability of measurements.
- NABL's primary focus is on assessing the technical competence of laboratories and their adherence to internationally recognized quality standards. It ensures that accredited laboratories consistently produce accurate and reliable test results.

Reference

1. Handbook Laboratory Quality Management System, WHO
2. National quality Assurance standard: https:// qps.nhsrcindia.org/national-quality-assurance- standards

CHAPTER - 21

LIMS, DATA ANALYSIS IN IHIP, OUTBREAK INVESTIGATION

LIMS and data Integration

To support surveillance and effectively manage outbreaks using geospatial information, it is imperative to digitalize all laboratory data. Ideally, the digitalization of laboratory information should be integrated into the Integrated Health Information Platform (IHIP). However, if IHIP is not available, any laboratory information system/data must be linked to IHIP to ensure seamless integration.

For efficient and accurate information dissemination to laboratory staff, clinicians, patients, and program managers, the computer-based information management system known as Laboratory Information Management System (LIMS) for Integrated Public Health Laboratories (IPHLs) is crucial. LIMS plays a vital role in helping laboratories adhere to quality standards, reduce transcription errors, minimize turnaround time from specimen receipt to result reporting, and ultimately improve patient outcomes.

There are two common types of LIMS:

LIMS within a Hospital Information System (HIS): This type of LIMS primarily serves as a tool to capture results and a few essential data elements within the existing hospital information system.

Stand-alone LIMS: This dedicated system encompasses most of the components mentioned above and supports all laboratory functions independently.

In both cases, the goal is to ensure the delivery of accurate and complete information efficiently, benefiting all stakeholders involved in the laboratory processes.

A good LIMS should be able to:

1. Track samples from collection to reporting.
2. Report test results for patient care (via HIS, email, SMS, etc.)
3. Collect, store, archive and analyze laboratory data.
4. Report analyzed data to district and state administration, Ministry of Health & Family Welfare (MOHFW)
5. Inventory management (kits and reagents etc.)

The registration for laboratory, in DHs lacking digitalized registration system will be done by a

DEO into the Laboratory Information Management System (LIMS) directly. Since most of the equipment is automated and linked with a computerized system so the same DEO can be utilized for compiling the reports.

The sample dispatch time should be recorded electronically in the LIMS and monitored till the report is generated and delivered to patients.

Reports from hub to spokes can be sent through LIMS or other electronic modes. A record for the same needs to be maintained both at hub and spoke. Patients can collect printed reports from the institution where the sample was taken. The intimation that the reports are ready will be sent through SMS to the patient along with the link for downloading the soft copy of the reports, wherever possible. This service may be in-addition to the right of the patient to receive the hard copy of reports where the sample was submitted.

The data integration can be through LIMS to monitor the data flow under various programs, facilities, departments to feed in to the IHIP platform for coordinated public health action.

Following stages but not limited to other processes and protocols as part of the laboratory network:

(a) Patient registration

(b) Sample collection

(c) Sample storage

(d) Sample transportation

(e) Sample processing

(f) Result entry

(g) Result validation and final Report Submission

(h) Recollection of samples

(i) Retesting of sample

Advantage of LIMS

Placing LIMS having several importance

- It reduces the transcription errors & faster processing with direct instrument uploads.
- Real time control of data quality with built in QC criteria.
- Direct report generation meeting specific patient requirements.
- Direct electronic reporting to clients or direct client access to data.

Principles of data analysis and its usage in surveillance and outbreak investigation

Learning Objectives:

Define data analysis and understand its significance in health decision-making. Identify various types of data analysis and develop an analysis plan. Apply data analysis techniques for surveillance and outbreak investigation.

What this module Cover?

Introduction to Data Analytics and Analysis in Health Systems

Principles of Data Analysis

Types of Data Analysis and Their Applicability Steps in Data Analysis Data Visualization with Hands-on Activities

Introduction to Data Analytics and Analysis:

"Data analytics" is used to describe statistical and mathematical data analysis that clusters, segments, scores and predicts what scenarios are most likely to happen using the data. Data analysis is a part of described as a method of cleaning, transforming, and modeling data into evidence for better health decision-making. The objective of Data Analysis is to obtain useful information from data to encourage evidence-based decision making.

In the field of public health, data analysis plays a vital role in surveillance and outbreak investigations. Laboratory technicians are crucial contributors to these activities as they generate, process, and analyze data obtained from various sources. This chapter aims to provide laboratory technicians working in health facilities with an understanding of the principles of data analysis and its significance in surveillance and outbreak investigation. By applying these principles effectively, laboratory technicians can contribute to

identifying and controlling health threats promptly and efficiently.

Principles of Data Analysis:

Data Quality: Laboratory technicians must ensure data accuracy, completeness, and reliability. Implementing quality control measures, following standardized protocols, and maintaining documentation are essential to generate reliable data for analysis.

Data Cleaning and Preprocessing: Before analysis, data often requires cleaning and preprocessing, which involves handling missing values, outliers, and inconsistencies. Laboratory technicians should be familiar with techniques such as data imputation and outlier detection to ensure data integrity.

Data Exploration: Exploratory data analysis techniques, such as descriptive statistics, data visualization, and graphical representation, aid in understanding the characteristics and patterns within the data. Laboratory technicians should be proficient in using statistical software and visualization tools to explore data effectively.

Statistical Analysis: Statistical methods enable laboratory technicians to quantify relationships, test hypotheses, and make inferences from the data. Basic statistical techniques, such as t-tests, chi-square tests, and regression analysis, are commonly used in public health data analysis.

Time-Series Analysis: Many surveillance systems rely on time-series analysis to detect temporal trends and seasonality. Laboratory technicians should be familiar with techniques like moving averages, autoregressive integrated moving average (ARIMA), and exponential smoothing to analyze temporal data.

Spatial Analysis: Geographical information systems (GIS) and spatial analysis techniques allow laboratory technicians to identify clusters, spatial patterns, and hotspots of disease occurrence. Understanding spatial epidemiology is essential for analyzing data with a geographic component.

Types of Data Analysis

There are four types of data analysis.

Descriptive Analysis: With descriptive analysis, we evaluate and define the characteristics of a data. Descriptive analysis, when combined with visualization provides us with a complete construct of data. In the descriptive analysis, we use with the past data to illustrate conclusions and present data as dashboards. of Key Performance Indicators.

Examples include creating performance dashboards

Predictive Analysis: With predictive analysis, we determine the outcomes of diseases, and hence the resource utilization, which is based on the analysis of the historical data. It makes use of descriptive analysis to generate predictions about the future. Predictive analysis is a complicated form of analysis and needs a large amount of data and a skilled workforce.

Examples include epidemiological models.

Diagnostic Analysis: It can be called as descriptive analysis in depth and is used to analysed anomalous patterns in data might contribute towards the poor performance of an intervention. With diagnostic analysis a system can reduce latency in logistics especially in drugs and vaccines' supply chains. Flaws in IEC activities can also diagnosed.

Prescriptive Analysis: Prescriptive analysis blends insights from all other analytical techniques. It is described as the final frontier of data analysis. Financial entities like NHM can leverage the power of this technique to increase their resource utilization.

Clinical Decision support systems use this form of analysis.

Uses of Data Analysis in Surveillance and Outbreak Investigation:

Early Detection and Reporting: Data analysis facilitates the early detection of unusual disease patterns,

enabling prompt reporting to public health authorities. Laboratory technicians should be vigilant in monitoring trends and abnormalities in the data they generate.

Trend Analysis: By analyzing historical data, laboratory technicians can identify long-term trends in disease occurrence, assess the impact of interventions, and predict future disease burdens. Trend analysis aids in strategic planning and resource allocation.

Identification of Outbreaks: Data analysis helps laboratory technicians recognize outbreaks by identifying significant increases in disease incidence or clustering of cases. Timely identification allows for rapid response and implementation of control measures.

Outbreak Detection and Response:

Signal Detection: Data analysis techniques, such as statistical algorithms or syndromic surveillance systems, can identify signals of potential outbreaks.

Cluster Analysis: Spatial and temporal analysis can help identify clusters of cases, facilitating targeted interventions and control measures.

Disease Mapping: Geographic information systems (GIS) enable the visualization of disease patterns and assist in identifying high-risk areas.

Source Identification: During outbreak investigations, data analysis assists in identifying the source of infection through epidemiological and laboratory correlation. Laboratory technicians play a crucial role in analyzing isolates and conducting genetic sequencing to establish links between cases.

Evaluation of Control Measures: Data analysis allows for the evaluation of the effectiveness of control measures implemented during outbreaks. Laboratory technicians can contribute by monitoring changes in disease incidence or testing the susceptibility of pathogens to specific interventions.

Steps involved for data analysis:

Data Collection: Gather relevant data related to surveillance or outbreak investigation. This can include patient records, laboratory test results, demographic information, and any other data sources that might provide valuable insights.

Data Cleaning: Review and clean the collected data to ensure its accuracy and consistency. Remove any duplicate entries, fix formatting errors, and address missing or incomplete information.

Data Organization: Organize the cleaned data in a structured manner, such as using spreadsheets or a database. Categorize the data into meaningful groups based on variables of interest, such as symptoms, geographic location, or time periods.

Data Exploration: Start exploring the data to get a better understanding of its characteristics. Calculate basic statistics, such as frequencies or percentages, to identify patterns or trends. Visualize the data using graphs or charts to make it easier to interpret.

Data Analysis: Apply appropriate statistical techniques to analyze the data. This could involve comparing groups, calculating rates, or performing trend analyses. The goal is to identify any significant associations, trends, or abnormalities that could indicate a surveillance issue or outbreak.

Interpretation: Interpret the results of the data analysis in the context of the surveillance or outbreak investigation. Look for patterns or outliers that might indicate the presence of a disease or the spread of an outbreak. Consider other factors, such as population characteristics or environmental conditions, that could contribute to the findings.

Reporting: Prepare a clear and concise report summarizing the data analysis findings. Include any important observations, trends, or recommendations based on the results. Use visual aids, such as tables or graphs, to enhance the clarity of the report.

Communication: Present the findings to relevant stakeholders, such as public health officials or medical officers or CMHO. Explain the key findings, provide necessary context, and address any questions or concerns they may have. Collaborate with other professionals to ensure the effective use of the data for surveillance and outbreak response.

It's important to note that the specific steps and techniques used in data analysis may vary depending on the nature of the surveillance or outbreak investigation, the available data, and the resources and expertise of the health facility.

Data Visualization

- Visualization is the conversion of data into a visual or tabular format

Types of Exploratory Data Analysis (EDA) are enlisted below:

Variable Groups	Type of EDA	Examples
1 categorical + 1 quantitative	Bar plot	IMR against gender or Distribution of blood types among different age groups
2 quantitative	Scatter plot	Income vs Health Expenditure / Laboratory Test Results vs. Patient Age
2 or three categorical + 1 quantitative	Stacked bar plots	Expenditure in public vs private
1 categorical + multiple quantitative	Multiple bar plot or stacked 100% column	Components of OOPE
Hierarchical data	Treemap or Bubble maps	Mortality rate by states

CHAPTER - 22

LABORATORY RECORDING

LAB RECORDING

I. Lab request

II. Registration

III. Statistics

I. LAB REQUEST:

How to request the lab test?

After the medic examines the patient, if he/she needs other examination from the laboratory how can he/she do? Using the lab request card is the suitable way that medic can ask lab technician for the test he/she needs. The result from the lab will also return to the medic through this request card.

A lab request card should provide sufficient information of the patient especially name, age, sex and address to keep in record and to prevent confusion of the specimens. The card must also state the type of specimen and the test required by the medic. Some other information of patients such as their suspected diseases and their clinical signs might be useful for the lab.

The lab request card is accompanied either with the specimen or the patient himself to the lab. Numbering of each specimen by laboratory is the way to make the lab – work run easier than using the patient's name. So provide some room for labeling.

The medics who request the test should also sign their name on the card: if any problem occurs the lab technician knows who to refer to. Also the lab technicians should sign on the card, as they are responsible for their results.

Example of lab request card:

Front side	Back side
LAB REQUEST FORM Date.. ... Pt. No.....Lab No... ... Name... ....Age... ....Sex..... Address... 3...Specimen/Test..... Information..... ..... Result... ...; ... parasitised.	Further Information / Result

Note: Pt. No = Patient Number.

Lab No = Lab Number.

Age: yr = year / ys = years / ms = months / ds = days, or you also can use the proportion as 1/12 = 1 month, 1/30 =1 day (example: 2 years 4 months = 2 4/12) Sex: F = female, M = male or ♀=female ♂= male

Test: M/S = malaria smear, UA urine analysis.

II. REGISTRATION:

After each lab examination is done, it is necessary to register all information with lab result to the register book. Then the card will be returned to the medic. Whenever we need to recheck any result or look back to any information, we can find it from this register book. The same tests should be registered in the same register book.

II a. Example of a malaria registration:

In the register book, you should have these following information:

Date	No	Name	Age	Sex	Address	C/O	Group	Result	Sign
5.5.24									
6.5.24									
7.5.24									

C/O: C = Camp / O = Other (outside the camp)

Group: A = < 5 years old

B = ³ 5 years old

Note: The classification of aging group depends on NGO's policy.

II b. Example of a sputum examination registration:

In the register book, you should have this following information:

No.	Name	Age	Sex	C/O	Date	TB Result	Paragonimus Result
1	Mg Mg	23y	M	C	10.5.24 11.5.24 12.5.24	NF NF NF	NF NF NF
2	Htee Oo	30y	M	O	20/5/24 21/5/24 22/5/24	AFB + AFB++ AFB++	NF NF NF

Every patient should have 3 sputum samples checked

You must do 1 slide per 1 sputum sample

1 PATIENT = 3 SLIDES

III. STATISTICS:

From the register book, you may check the quantity of each lab test done in a certain time; such as the number of positive malaria slides found last month or the number of AFB tests done in camp # 3 last year. In general, all the numbers are collected on a monthly basis. These figures should be continuously kept in record. We call this process statistics

Statistics are useful in the control and prevention of diseases. Lab statistics also can give you the amount of test done in each period around the year and the number of positive cases found from all suspected cases. It also helps you to estimate the quantities of each test in the future and this also helps you in ordering supplies.

Every month you must give a report of the number of examinations you made during the month.

Statistic Form:

Daily or weekly check the register book and count the number in each group.

At the end of each month, you must fill in the following statistic form correctly.

Month / Year: _____ **Camp: _____**

MALARIA

	A < 5 years old		**B ³ 5 years old**		**A + B TOTAL**		**PW (*)**
	Camp	**Others**	**Camp**	**Others**	**Camp**	**Others**	
PF							
PV							
PF + PV							
PFG							

Total Pos							
Total Neg							

Total Slides							

(*): PW = Pregnant women

SPUTUM

	Total Slides				Total Positive Slides				Total Patients				Total Positive Patients			
Origin	**Camp**		**Others**		**Camp**		**Others**		**Camp**		**Others**		**Camp**		**Others**	
Group	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
AFB																
Paragonimus																

A: less than 5 years old (< 5 ys) B: more than or equal to 5 years old (³ 5 ys)

In case there is a lot of work in your lab, doing daily statistic can help you to fill monthly statistic forms more easily and also can help you to see the trend in malaria situation day by day.

If you classify more than 2 aging groups, you need another table to cover all data.

III a. Malaria statistics

Count these numbers from the malaria register book and fill in the form:

- The total number of the slides.
- The number of positive slides.
- The number of negative slides.
- The number of PFT (Plasmodium falciparum trophozoite) slides.
- The number of PVT**(*)** (Plasmodium vivax trophozoite) slides.
- The number of mixed (P. falciparum+P.vivax) slides.
- The number of PFG (Plasmodium falciparum gametocyte) slides.

(*)Remark: It is not necessary to count P. M and P.O separately. They can be added to the PV (as Non-PF malaria).

III b. Sputum examination statistics

For AFB and Paragonimus tests, you need to count these numbers from the register book and fill in the form:

- The total number of slides.
- The total number of positive slides.
- The total number of patients.
- The total number of positive patients.

CHAPTER - 23

INVENTORY / ORDER

The INVENTORY SYSTEM is needed to control the amount of materials you are using in the laboratory. This system will help you to continue your work smoothly.

You should record all of materials you have in your inventory book.

There are 2 kinds of materials: **1. Permanent material**

2. Consumable material

1. **Permanent material:** It is the material that can be use for its lifetime if it is not broken and you hardly need to order it.

   List of permanent materials used in this manual is

Microscope	Cylinders
Pipettes	Wash bottles
Drop bottle	Funnels
Staining basins	Staining racks
Slide drying racks	Buckets
Basins	Trays
Burning lamps	Timers
Slides boxes	Test tube
Centrifuge	Tube rack
Haematocrit centrifuge and/or	Haemoglobin meter

   If one of these things is missing or broken, request a new one when you make the laboratory order.

2. **Consumable material:** It is the material you consume for the lab tests and need to be ordered to ensure that you will have enough to work in the coming period.

   List of consumable materials for each laboratory examination:

For Malaria Exam.	For Sputum Exam.	For Blood Transfusion
Giemsa stain	Carbol fuchsin	Antiserum–A & antiserum .
Absolute Methanol	Methylene blue	Microscopic slides
Microscopic slides	Acid–alcohol 3 %	Blood lancets
Blood lancets	Burning alcohol	Cotton ball
Xylene	TB slides (with frosted end)	Rubbing alcohol or savlon
Immersion oil	Immersion oil	HIV–test kit
Lens paper	Lens paper	Hep–B test kit
Filter paper	Filter paper	Sterile syringe
Silica gel (desiccant)	Silica gel (desiccant)	Sterile 18 or 21 G needle
Tissue paper	Tissue paper	Disinfectant
Cotton ball	Cover slips	
Rubbing alcohol or savlon	Specimen container	
	Masks	
	Matches	

List of Consumable Materials for administration in the laboratory:

- Statistic forms
- Lab Request forms
- Quality Control forms
- Register books
- Pens
- Pencils

THE STOCK AND ORDER

In order to maintain the inventory effectively, you need to do the stock and order of materials.

What is the stock – order?

- The stock is the list of the quantities of consumable materials and some permanent materials, which can easily be broken (pipettes, oil drop bottle, and timer..) that you have in the cupboard of your laboratory.
- The stock is the materials that you currently have and which had NEVER been USED.
- The order is the list of the quantities of materials and reagents you need for the next period.
- You register the stock and the orders in the laboratory stock – order form when you make your laboratory order and give it to the in charge.

How to do a laboratory stock – order?

1. Check the stock at the END of EACH MONTH or AS ARRANGED with your in charge.
2. NEVER wait until the amount become "0" before ordering.
3. NEVER order without checking what you have in the stock.
4. ORDER ENOUGH for the number of months decided including the minimum necessary stock for emergency situation.
5. GIVE the laboratory ORDER to the IN CHARGE.

ADVICE FOR MAKING YOUR LABORATORY STOCK - ORDER

Estimate the amount of each material needed for each test using the statistics of the test done either in previous month or in the same month of the previous year.

Material	Amount per pack	Amount used per test	Approximated No. of tests / pack	Remark
Immersion oil	500 ml / btl	0.15 ml / slide	3000 slides / btl	
Giemsa stain	450 ml / btl	0.5 ml / slide	800 slides / btl	10% dilution
Abs. Methanol	2.5 liter / btl	100 ml / time & 1 time / week	25 weeks / btl	**Rainy season**
Abs. Methanol	2.5 liter / btl	100 ml / time & 1 time / 2 weeks	50 weeks / btl	**Dry Season**
Carbol fuchsin	450 ml / btl	5 ml / slide	80 slides / btl	
Methylene blue	450 ml / btl	5 ml / slide	80 slides / btl	
Acid–alcohol	450 ml / btl	10 ml / slide	80 slides / btl	
Burning alcohol	450 ml / btl	5 ml / slide	80 slides / btl	
TB slides	72 slides / box	1 slide / test	70 tests / box	
Tissue paper	1 roll		200 slides / roll	
Filter paper	100 pieces / box	50 slides / piece	5000 slides / box	To filter the water
Silica gel (Desiccant)	1kg / bag	1 kg / microscope for 6 month	1bag / microscope	For 6 months
Blood lancet	200 pieces / box	1 piece / test	200 tests / box	
Malaria slides	72 slides / box	1 slide / test	70 tests / box	Slides can be re-used

Other suggestion note:

Absolute Methanol:

* Don't throw away the old methanol. Place this methanol into a clean bottle with label "Old Methanol".

What could we do with the old methanol?

- After washing the malaria slides with soap powder and water, leave them to dry and clean them with a piece of gauze containing this old methanol.
- You can use this old methanol to wash the glass pipettes or any old bottle containing stain in order to remove the stain that remains.
- You can also use it to renew from time to time the wide–mouth pot containing sand & alcohol in the TB room.

Blood Lancets:

For each malaria slide done, you need 1 blood lancet. If 600 malaria slides done during 3 months correspond with the use of 3 boxes of blood lancets. If you do the determination of 60 haemoglobin or haematocrit per month in your lab you need to add 1 box of lancets to perform this exam for 3 months.

The total number of boxes of blood lancets should be: (3+1) = 4 boxes of blood lancets for 3 months.

The number of blood lancets you need to order should be multiply by 1.5 in order to have a safety margin.

As the above example the number of the boxes of blood lancets should be: (4 x 1.5) = 6 boxes of blood lancet for the next 3 months.

Malaria Slides:

The quantity of boxes of malaria slides ordered should be the same as the quantity of boxes of malaria slides used each month.

Laboratory Request Forms:

The number of the lab request forms needed correspond to the number of the request forms used during the past months.

APPENDIX 1: LEARNING MATERIALS

Color plates used in this training manual can be purchased at the following addresses:

1. Microscopic Diagnosis:

   Bench Aid Series
   - No.1 Malaria
   - No.3 Filariasis
   - No.5 Meningitis – AIDS – Gonorrhoea – Syphilis – TB - Leprosy
   - No.7 Blood: Normal – Anaemias – Infections - Leukaemias

   Available from Tropical Health Technology, 14 Bevills Close, Doddington, March, Cambridgeshire, PE15 OTT, UK.

2. Bench Aids for the Diagnosis of Malaria, Set No. 1 plates 1-4 and Set No. 2 plates 5-8. Illustrations by Yap Loy Fong. These teaching Aids can be obtained from:

   World Health Organization
   Distribution and Sales Service
   1211 Geneva 27
   Switzerland

   or from

   HMSO
   P.O.Box 276
   London SW8 5DT
   UK

3. Plasmodium parasites pictures were provided by SMRU and by the Royal Perth hospital web site:

   http://www.rph.wa.gov.au

APPENDIX 2: REAGENTS AND EQUIPMENT

The quantities of laboratory supplies available should be sufficient to maintain the lab–work for the period of time between each supply distribution. The recording of all supplies remaining and received should be done regularly, at least before ordering the new supplies.

Reagents used in this manual are:

Reagent	Use
Burning alcohol	for spirit lamp and other flaming
Carbol fuchsin	AFB staining
Concentrated Hydrochloric acid (HCL)	making of acid-alcohol
Ethanol 95%	making of acid-alcohol
Giemsa stain	blood staining
Immersion oil	examination with X100 objective
Hypochlorite	disinfectant for waste specimen and others
KOH solution 3% (diluted from 30% KOH)	dissolves the sputum
Methanol absolute	fixing thin blood films
Methylene blue	AFB staining
Rubbing alcohol	cleaning and disinfecting of skin

Equipment used in the examinations described in the manual:

Alarm clock (in minute)	timing for staining
Beaker 50 cc, 100 cc	for mixing of the stain
Lancet	for taking capillary blood
Brush, soft hair	for microscope cleaning
Clay sealer	for plugging of microhaematocrit tube
Cleaning gloves	for washing
Conical tube 12–15 cc	for specimen centrifugation
Cotton wool	for skin cleaning and making swabs
Square coverslips	for wet preparation
Cylinder 100 cc	for measuring of reagents
Dropper bottle, brown glass, 40–65 cc	for containing of reagents

Filter paper 24 cm diameter	for filtration of the water
Filter 12.5 or 15.0 cm diameter	for filtration of the stain
Forceps	for holding of slide
Glass lid bottle, 60 – 100cc	for containing of Giemsa stain
Glass rods	for hanging of the slides duringstaining
Hand centrifuge	for sedimentation
Label sticker	for specimen labeling
Lab request form	for examination request
Lens paper	for cleaning of microscope lens
Matches	for burning
Microhaematocrit tubes, heparinised	for collection of capillary blood
Microscope slides	for general microscopic examination
Microscope slides, frosted ended	for sputum examination
Needles 20 – 24 G	for taking Giemsa stain from the bottle and for venous blood collection
Pipette, graduated 10cc	for measuring Giemsa
Rubber bulb for pipette	for sucking of reagent by pipette
Silica gel	for absorption of the moist
Syringe 5 cc, 10 cc	for preparing in Giemsa staining and for venous blood collection
Slide box	for keeping the examined slides
Slide drying rack	for hanging slide to be dry
Specimen container, wide-mouth pots with screw cap	for specimen collection
Spirit lamp	for burning and flaming
Staining tray	for staining
Tube brush	for washing tubes

REFERENCES

- Basic Laboratory Methods in Medical Parasitology, World Health Organization Geneva, 1991 Jeffrey & Leach, Atlas of Medical Helminthology & Protozoology
- Manual of Basic Techniques for a Health Laboratory, World Health Organization Geneva, England, 1980.
- Monica Cheesbrough, Medical Laboratory Manual for Tropical Countries, volume I and II, Tropical Health Technology, Cambridgeshire/ Butterworth-Heinemann, Oxford 1994
- Radomyos Prayong, Tangtrongchitr Anchalee, Looareesuwan Sornchai, Chongsuphajaisiddhi Tan, Atlas of Medical Parasitology with 456 colour illustrations, Mahidol University, 1992.
- Pracrtical Haematology Laboratory Manual : Prepared By: Deemah Al Dabbagh Demonstrator CLS Department
- Reticulocytes - Reticulocyte count Pictures & Images. (n.d.). AllRefer Health Retrieved December 30, 2011, from http://health.allrefer.com/health/reticulocyte-count-reticulocytes.html
- Lewis, S. M., Bain, B. J., Bates, I., & Dacie, J. V. (2006). Dacie and Lewis. practical haematology
- (10th ed.). Philadelphia: Churchill Livingstone/Elsevier.
- Alessio Panza, Migrants: HIV Testing and Counseling a manual for IOM counselors, International
- Organization for Migration (IOM), Bangkok, 1994
- analis laboratorium kesehatan: THICK AND THIN BLOOD SMEARS FOR DETECTION OF
- MALARIA, BABESIA, AND OTHER BLOOD PARASITES. (n.d.). analis laboratorium kesehatan. Retrieved December 30, 2011, from http://analislaboratoriumkesehatan.blogspot.com/2010/03/thickand- thin-blood-smears-for.html

Annexure -1

SETTING UP PUBLIC HEALTH LABORATORY

Principles of Laboratory Design

1. The laboratory shall have adequate space allocated designed to ensure the Quality, safety and efficacy of the service provided to the users and the health and safety of laboratory personnel, patients, and visitors.

2. The laboratory needs to be compliant with mandatory regulations like Biomedical Waste Management Rules, 2016, Liquid waste management, Fire Safety, potable water, HIV/AIDS prevention and control Act (with mandatory provision of pre- and post-testing counselling), Occupational Health and Safety Management System (OHSMS) and other such regulatory requirements for running a laboratory.

3. **Clean electric power supply:** Electrical installation must be done carefully to ensure proper distribution of electric load along with load balancing to various equipment and installations in a laboratory. Automatic voltage regulators which regulate fluctuating input power voltage and maintain constant output voltage should be provided. Adequate electric points on the various walls (at < 1.5 m height from the floor) needs to be ensured for easy connection. Use of explosion proof plugs, plug connector and socket is essential to ensure safety against explosion. All equipment must have separate connections with electrical points for the safety of the equipment. New electrical appliances should have a minimum 3-star rating from the Bureau of Energy Efficiency or equivalent recognized organization to minimize the energy input.

4. The laboratory should have a round-the clock power back-up option, a UPS connection should be provided with critical equipment to ensure uninterrupted testing in case of a power cut.

5. The laboratory should have an arrangement for round the clock potable piped water supply with a provision to store at least three days of water requirement.

6. There should be separate Air Handling Units (AHUs) for Mycobacteriology and Virology laboratories. AHUs are required to maintain negative pressure in these highly infectious areas. However, a positive pressure needs to be maintained for other areas to prevent the spread of infectious spore/viruses to the rest of the laboratory. Thus, installation of Heating Ventilation and Air conditioning (HVAC) system is sufficient in other areas of core testing facility. The category of HEPA filter H13-14/99.7% efficiency with air flow speed 25-35 FPM (feet per minutes) can be used for ventilation. Ductless AHU can be preferred since it reduces the maintenance cost, growth of microbes and frequency of cleaning.

7. Also, air changes are critical for controlling airborne organisms. It is suggested that the air changes of critical areas like Mycobacteriology and Virology will be like that of an ICU that is ideally 12-15 air exchange and out of these, six should be fresh air exchanges per hour. The specifications to be maintained will be humidity 45-65%, temperature 23±2, positive and negative pressure - 2.5Pa.

8. Since this laboratory is an integrated laboratory including Bacteriology, Mycobacteriology and Virology/Molecular Diagnostic sections, entry to the laboratory needs to be regulated through access control to ensure Biosafety and Biosecurity. The high containment areas (bacteriology,

mycobacteriology, and virology) will be set up in the farthest corner of the facility (These areas are demarcated in the sample layout). There shall be adequate provision for waste segregation and disposal in the IPHL and disposal for highly infectious materials shall be in accordance with latest biomedical waste disposal guidelines of the Central Pollution Control Board (CPCB) of Government of India. Liquid waste generated in the laboratory needs to be connected to an Effluent Treatment Plant (ETP) of the hospital through a dedicated outlet.

9. The following specific design requirements for a BSL-2 Lab need to be in-built in the design of the laboratory.

   a) Doors should have access controls (lockable door if housing restricted agents) and a sink must be located near the exit door.

   b) A handwashing sink should be present.

   c) Bench tops should be impervious to water and resistant to moderate heat and organic solvents, acids, alkalis, and chemicals used for surface decontamination.

   d) No fabrics or carpeting allowed in the lab.

   e) Biosafety Cabinets (BSCs) should be positioned such that fluctuations in air supply and exhaust or the operations of equipment do not alter the performance standard of the cabinet.

   f) Eyewash station should be readily available. Autoclave should be available in the facility. Negatively pressurized sections of the laboratory (e.g., Mycobacteriology, Virology lab) should have 100% exhaust.

   g) The laboratory should be equipped with fire/emergency exits along with provisions for appropriate storage of inflammable materials and appropriate class of extinguisher (class BC or ABC).

Setting up Sample Collection Area

- Sample receiving area should have Computer with scanner, printer, UPS, report for Sample acceptance and registration Report generation and delivery. Barcode generator, Barcode reader

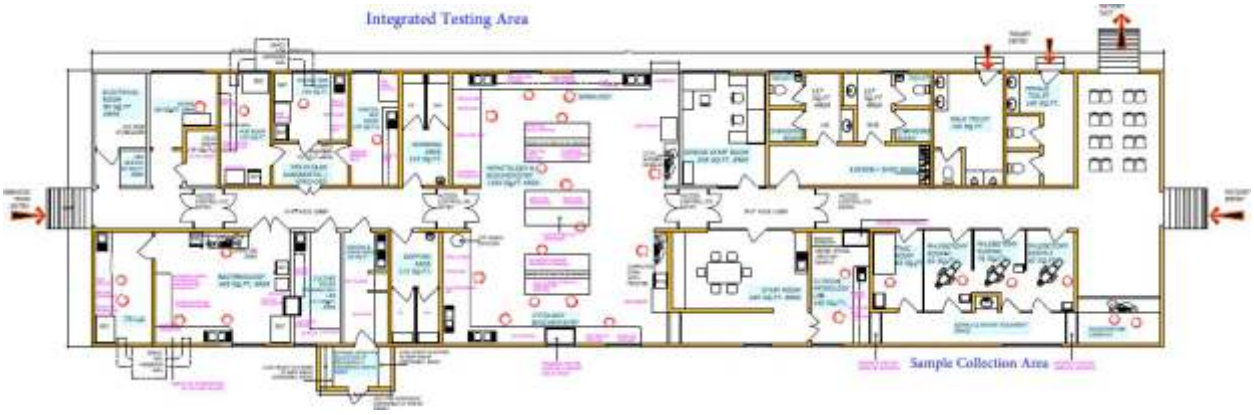


Figure 3: IPHL Layout (IPHL Guideline)
Source: https://nhsrcindia.org/sites/default/files/Guidelines%20on%20integrated%20public%20health%20laboratories.pdf

Setting up Change Room

Changing Room should be dedicated for male and female separately.

- Should have dedicated hooks for hanging the Cloths.
- Should have Racks for storage of fresh personal protective equipment (PPE).
- Should have appropriately sized PPEs.

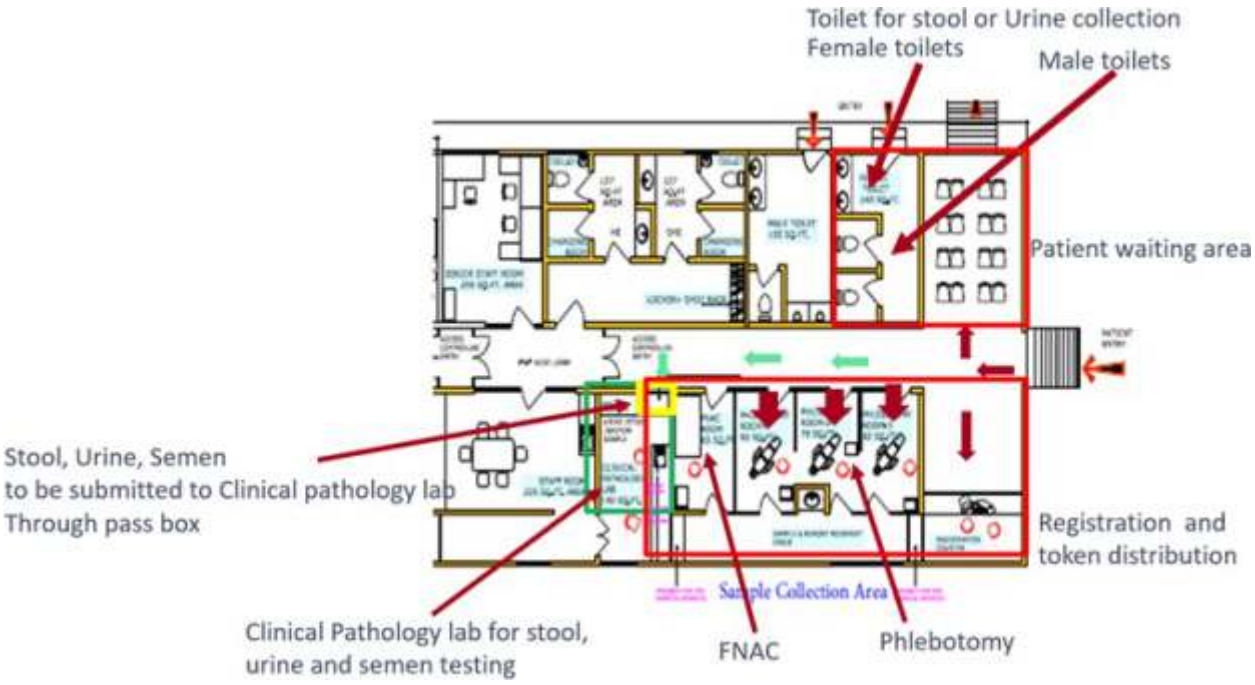


Figure 4: Patient Flow-Sample Collection Area (IPHL Guideline)
Source: https://nhsrcindia.org/sites/default/files/Guidelines%20on%20integrated%20public%20health%20laboratories.pdf

- PPE- Lab coat (S, M, L), Gloves (S, M, L), Respirators/ Masks (for appropriate use), Shoe Cover, eye protection
- There should be a dedicated locker to keep personal belongings.
- Shoe Rack
- Disinfectant

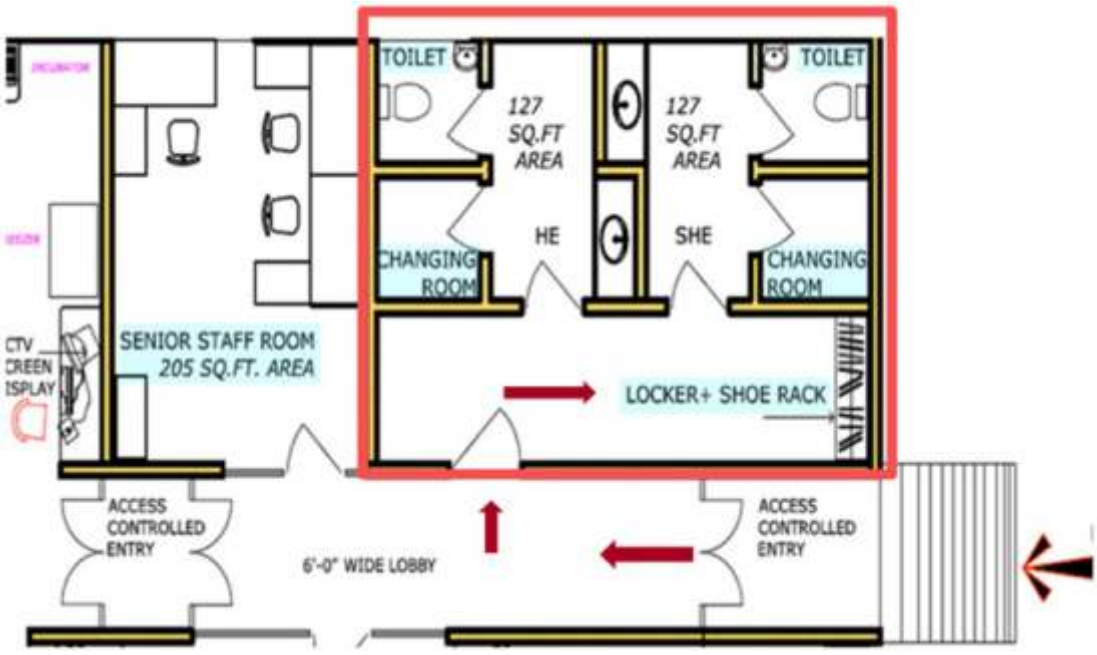


Figure 5: Setting Up Change Room (IPHL Guideline)
Source: https://nhsrcindia.org/sites/default/files/Guidelines%20on%20integrated%20public%20health%20laboratories.pdf

Setting up Clinical Pathology Lab

Sample receiving in Clinical pathology should have through pass box. Sample may be Urine, Stool, Semen, and sputum. Urine samples are collected for microscopy, chemistry, or pregnancy tests.

Stool samples are collected for microscopy for ova, cysts and parasites, occult blood, Hanging drop for cholera, Rapid test for Cholera. Semen Analysis by Microscopy. Required Equipment is clinical pathology laboratory are: Binocular Microscope, Urine analyzer, Centrifuge, Bunsen burner with gas supply.

Setting up Hematology Lab

Samples received in hematology laboratory for the microscopy of peripheral blood smear for blood cells & haemoparasites (malaria/filaria), Complete hemogram & cell count, Blood grouping, cross matching, Coombs and hemoglobinopathies, Coagulation profile, Bone marrow examination. Equipment should be placed on the tabletop. Required equipment in this section are Binocular Microscope, Automated Cell Counter (3 part/5 part) with nucleated RBC flag, Automated Coagulometer, Automated ESR analyzer, Hemoglobin HPLC machine (variant analyzer).

Setting up Biochemistry lab

In the biochemistry lab, Quantitative analysis of routine biochemical parameters and special chemistry parameters (including hormones) are expected to be performed. Required equipment in these sections are Automated Biochemistry analyzer, ISE based Electrolyte analyzer, Automated Hormone, Immunoassay analyzer.

Setting up Serology lab

In this section ELISA for Dengue, Chikungunya, Leptospirosis, Scrub typhus, Hepatitis A & E etc, Rapid card tests for Malaria, Hepatitis B & C, HIV etc. are expected to be performed. Equipment required in this section area, ELISA reader and washer and VDRL rotor/ Shaker.

Setting up Cytology lab

Fine-Needle Aspiration Cytology (FNAC), PAP smear examination CSF & body fluid cytology are tests usually performed in cytology section. Binocular microscope and Centrifuge are the main equipment of this section.

Setting up Microbiology lab

In the IPHL, microbiology lab comprised of two sections, general microbiology lab and mycobacteriology/ TB lab. This section is expected to be ventilated unidirectional air circulation through Heating, Ventilation and Air-Conditioning (HVAC). In this section Microscopy, Bacterial Culture & Antimicrobial susceptibility testing for clinical samples, microbiological analysis of water (H_2S test for screening, Coliform presence absence (PA) test for confirmation are expected to be performed. Equipment in this section comprised of Binocular Microscope, Incubator, Automated blood culture, Automated bacterial ID/AST system, Biosafety Cabinet Class II A2 (model conforming to NSF standards), Bunsen burner with gas supply. In the mycobacteriology lab microscopy for acid fast bacilli (AFB) and nucleic acid amplification test (NAAT) for mycobacteria are performed. Equipment placed in this section are Binocular Microscope, Fluorescent Microscope, Biosafety Cabinet Class II A2 (model conforming to NSF standards), CB-NAAT, Bunsen burner with gas supply.

Setting up molecular biology/ Virology lab

Molecular laboratory divided into 3 sections/ cubicles. Nucleic Acid Extraction, Master mix preparation and PCR Room, Equipment may be placed as per use. The extraction room should have a Biosafety Cabinet, Nucleic acid extractor, Microcentrifuge, Micropipettes. Master mix Room should have PCR workstation and micropipettes. The PCR room should have a PCR workstation and real-time PCR machine. This section should also have Ventilated air Circulation through AHU/HVAC.

Setting up Media Room

Media preparation room is dedicated for all reagents and media preparation. This section should have electronic balance, Hot plate, Bunsen burner with gas supply and pH meter.

Setting up Washing and Sterilization Room

This area should have, Autoclave (One for clean items and one for decontamination of dirty items) Hot air oven, Exhaust fan.

Biosafety in Public Health Laboratories

Laboratory biosafety and biosecurity activities are fundamental to protecting the laboratory workforce and the wider community against unintentional exposures or releases of pathogenic biological agents.

This chapter briefs about principles of biosafety at different level of laboratories functional at different levels of public health system (district level, CHC lab, PHC lab and even HWCs)

General Principles of Biosafety

What is Biosafety?

- Laboratory biosafety" is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.
- The term "containment" is used in describing safe methods, facilities, and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained.
- **Biosecurity:** "Laboratory biosecurity" refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Elements of Containment

Persons working in the laboratory must follow the basic elements of containment which Include:

- Strict adherence to standard microbiological practices and techniques.
- Awareness of potential hazards among persons working with infectious agents or potentially infected materials
- Must be trained and proficient in the practices and techniques required for handling hazardous material safely.
- Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards.
- There are 3 major barriers to laboratory biosafety.
    - o **SOP, technique, Safety Practices:** This is primary barrier, when standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed.
    - o **Safety equipment:** These are the secondary barriers and include use of appropriate biosafety cabinets, safe centrifuges and appropriate Personal Protective Equipment (PPE) such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety

World Health Organization (WHO), LABORATORY BIOSAFETY MANUAL 4Th EDITION

glasses, or goggles as per the risk assessment.

- **Appropriate facility design and engineering features:** Appropriate facility design and engineering features
  - These are the tertiary barriers and provide protection to the laboratory workers, to persons outside the laboratory, and persons or animals in the community from infectious agents that may be accidentally released in the laboratory.

Biosafety Levels

Four Biosafety Levels (BSLs) are described based on combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. BSL-1 are basic teaching laboratories, BSL-2 are diagnostic services and research laboratories, BSL-3 are high containment special diagnostic services laboratories and BSL-4 are maximum containment laboratories to handle dangerous pathogens. Laboratory biosafety manual (3rd Ed), WHO, describes relative hazards of infective microorganisms by risk groups for laboratory work (Table 1). The classification of microbiological agents is based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

Table 1: Biosafety level and Risk group

Risk group	Biosafety level (BSL)	Laboratory type	Laboratory practices	Safety equipment	Organism Example
1	BSL1	Basic teaching, Research	Good Microbiologica lTechniques (GMT)	None; open bench work **Updated**: Involves wearing a face mask and no close contact	Viruses and bacteria, like Escherichia coli and chickenpox and many non-infectious bacteria
2	BSL2	Primary health services; diagnostic services; research	GMT plus protective clothing, biohazard sign	Open bench plus Biological Safety Cabinet (BSC) for potential aerosols	hepatitis A, B, and C, Lyme disease, Salmonella, measles, mumps, HIV, and dengue
3	BSL3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or primary devices for all activities	Mycobacterium tuberculosis, anthrax, many types of viral encephalitis, hantavirus, Rift valley fever, virus, malaria, Rocky Mountain spotted fever, and yellow fever virus.
4	BSL4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air	Ebola, Marburg virus, Lassa fever, Bolivian hemorrhagic fever, other hemorrhagic viruses

Table 2: Facility Design Requirement 4 biosafety level

Requirement	BSL1	BSL2	BSL3	BSL4
Isolation of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
• Inward airflow	No	Desirable	Yes	Yes
• Controlled ventilating system	No	Desirable	Yes	Yes
• HEPA filtered air exhaust	No	No	Yes/No	Yes
Double door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	No	Yes
Requirement	**BSL1**	**BSL2**	**BSL3**	**BSL4**
Anteroom with shower	No	No	Ye/No***	No
Effluent treatment	No	No	Ye/No***	Yes
Autoclave				
• On site	No	Desirable	Yes	Yes
• In laboratory room	No	No	Desirable	Yes
• Double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability***	No	No	Desirable	Yes

**Depending on agents used in laboratory,
***Glass windows, closed circuit television, two-way communication
References: WHO Laboratory Biosafety Manual, 3rd Ed, 2004

Risk Assessment

- Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory Associated Infection (LAI), and the probable consequences of such an infection. Following factors should be considered for conducting risk assessment:
- Risk group of the microbiological agent
- Pathogenicity of the agent and infectious dose
- Potential outcome of exposure
- Natural route of infection
- Other routes of infection, resulting from laboratory manipulations
    - o Direct contact – skin, mucosa or eye
    - o Parenteral – inoculation (needle or contaminated sharp), bite from infected Vectors
    - o Airborne – inhalation
    - o Ingestion
    - o Handling of laboratory animals

Risk Assessment Steps

There are 3 main steps of conducting risk assessment.

Step 1: Identify General Hazards: Mark off the known risk of hazardous agents, process, and equipment. Use following list as a tool to help complete the risk assessment u=in step 2.

Hazardous chemicals, substances, Biohazards		
Chemical/ Substances		Biohazards
Compressed gases- Flammable Compressed gases- Oxidizing Compressed gases- Toxic Compressed gases- inert Cryogenic material Organic peroxide Peroxide formers Self-reactive substances Water reactive substances Pyrogenic	Acute toxic chemicals Carcinogens Nano materials Reproductive toxins Simple asphyxiant Corrosive liquid DEA/ Controlled substances Specific organ toxicity Explosive Flammable liquids Oxidizers/reducing agents	Animal infection studies Large scale culture Risk group 2 pathogens Risk group 3 pathogens Plant pathogens Biological toxins Human blood, body fluid Cell culture Virus/ recombinant viral vector Transgenic plants/ Animals

Hazardous process or Equipment		
Explosion hazard Exothermic with potential for fire or excessive heat Acid bath Hazardous reaction or products Generation of air contaminants (gas/aerosol/particulates) Heating chemicals Large volumes Chemical transferrin	Hand/power tools Moving equipment or parts Electrical hazards Noise >85 db Hot surfaces Ergonomic hazard Needle/sharps Drying over/ furnace Centrifuge Working alone/ after house	Unattended reactions Respiratory hazard Vacuum/ pressure systems Refrigerators/ freezers Sterring and mixing devices. Laboratory microwave ovens Slip trip falls. Repetitive motions

Filed Hazard		
Foul weather Temperature extremes Darkness/ low light Altitude Smoke/ dust Wild animal/ insect Plant/ allergens	Vector born/ other endemicdisease. Hygiene/ waterborne/ food born illness. Falling objects Boating/swimming/water hazard Limited communication Remote area/ limited medical services	Lifting/ carrying Strenuous physical activity (e g long days, high stress) Driving/ operating a vehicle (eg tractor OSU or personal) Un even surfaces Heights

Shop/ Laser/Radiation		
Shop	**Laser**	**Radiation**
Aerial Lift Air compressor Crane Forklift Hot work Used/ New Oil	Class IIIb Laser Class IV laser	X ray machines Magnetic field (NMR. MRI) Radioactive material Unsealed source/ radionuclides Sealed source/ radionuclides Ultraviolet light/ Ifra red light

Step 2: Perform Risk assessment: fill out the risk assessment form as per suggested below in table.

Name:					
Rooms Associated:					
Person at Risk: □ Employee □ Student □ Public □ visitors □ other					
Task/ activity/ chemical	Significant hazard	Potential consequenceof hazard	Initial risk level	Control measures	Final risk level
Example: using hydrofluoric acid	Causes sever burn with delayedtissue destruction.Rapidly absorb through the skin. Causes tissue necrosis and bine destruction. Exposure may not be immediately visible or painful	Long term scaring, hospital stay, could be fatal	H	If a substitute is available, this would be the first potential control measure. If not other measures such as using HF in fume hood only, Proper PPE (double gloves, chemical resistant lab coats, closed toed shoes, long pants, face shield), proper singes, proper training, do not workalone or after hours and an exposure kit with fumes and calcium gluconate	M

Step 3: Action plan: Implement control measures and create standard operating procedures and training.

Once the control measures are identified, one should take steps in implementing the control measure into their process prior to beginning the work. As a suggestive model is given as below.

Exhibits: NIOSH has developed hierarchy of controls as amen of determining ho to implement feasible and effective control solution to exposures of hazard. Control methods at the top are potentially more effective and protective than those at the bottom. Following this hierarchy normally leads to the implementation of an inherently safer system, where the risk of illness or energy has been substantially reduced.

Hierarchy of Control

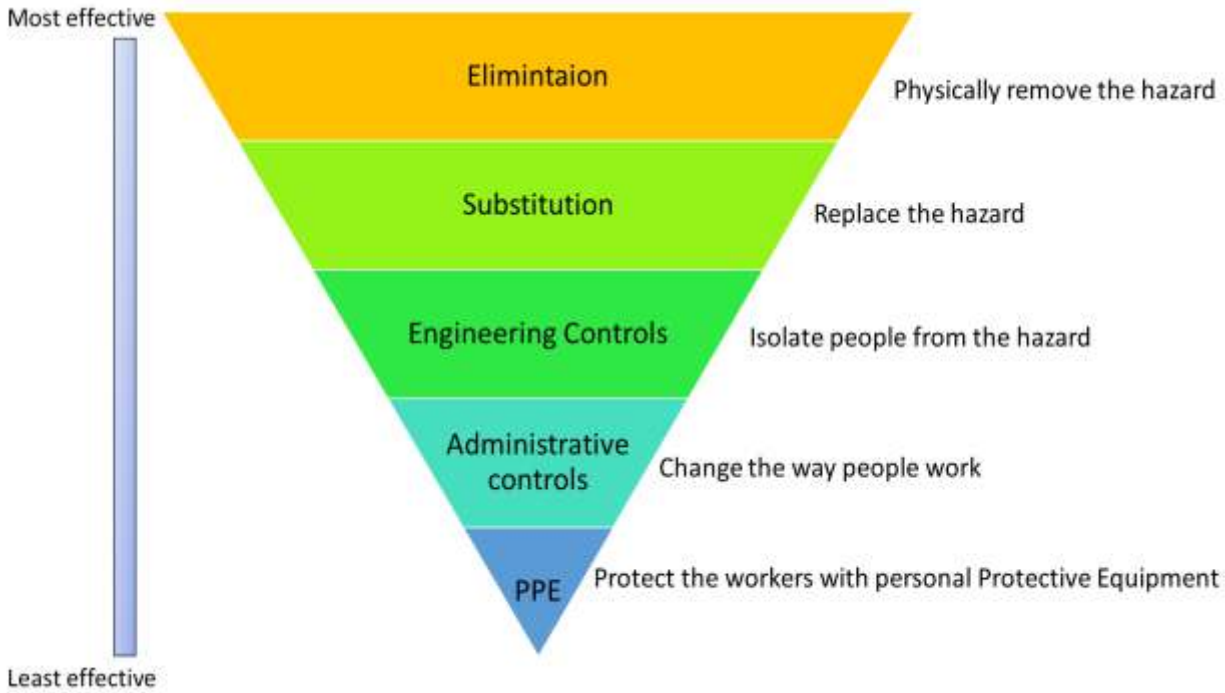


Table: Use this table to score your hazard or activity's risk while performing a risk assessment. Use “L” for low, “M” for medium and “H” for high while describing risk in your risk assessment tool.

		Risk Matrix		
Likelihood/Probability		Low	Medium	High
	High	Medium	High	High
	Medium	Low/ Medium	Medium	High
	Low	Low	Low	Medium
		Consequence/Severity		

Table: This table defines what a low, medium, and high risk is based on risk to people, environment, operation and reputation. Use this table to assist Risk matrix.

Consequence/Impact				
		People	**Environment**	**Reputation**
Severity	Low	No safety or health hazard present, first Aid injury, short term exposure to mild health effect.	Minor spill or emission/ slight effect	Department/ college level attention
	Medium	Medical treatment injury, chronic health effect, time loss<7 days, moderate health effect.	Controlled environmental release, minor and/or localized effect	Local level attention, OSU school level attention
	High	Severe heath effect, time loss >7 days, permanent incapacitation, or disabilityof fatality	Some permanent ecological damage, major effect, widespread ecological damage	State, National, and international level attention

This table defines, what a low, medium and high likelihood/ probability is for the risk matrix tool.

Likelihood/ Probability	Explanation
Low	Nearly unlikely to happen in the near future and no immediate action is needed
Medium	Moderate likelihood or likely to occur and actions should be taken to reduce orcontrol risk
High	More than likely to occur or high probability the risk will occur, immediate actionplan required

Biosafety Cabinets

A biosafety cabinet is a ventilated, enclosed cabinet, used during work on hazardous micro-organisms, protects both operator and environment from exposure to infectious aerosols generated during active handling of liquid materials.

- Some classes of BSC protect materials in use from cross-contamination.
- The BSC is the most important piece of equipment in a microbiology/ virology laboratory for the primary containment of biological hazards.
- When properly installed, used and maintained, BSCs will:
    - o Reduce laboratory-acquired infections.

Figure 6: Biosafety Cabinet
Source: https://www.escolifesciences.com/products/class-ii-biological-safety-cabinet/labculture-class-ii-type- a2-bsc#gallery

- Reduce cross-contamination of cultures.
- Protect the laboratory environment from hazardous microorganisms.

Safety Features of Biosafety Cabinet

Operators should monitor 3 features of biosafety cabinet routinely.

- High efficiency particulate air (HEPA) filters
- Airflow patterns
  - Air curtain at opening
  - Laminar flow of filtered air inside
- Exhaust system
- Filtered air exhausted.

HEPA filters: Constructed of paper-thin sheets of borosilicate fibers, pleated to increase surface area and affixed to a frame, 99.97% effective in removing particles as small as 0.3 μm in, diameter, highly effective in trapping bacilli (each ~ 2–10 μm long and 0.4 μm wide), After filtration, microbe-free air is exhausted from the BSC. (Figure 7)

Figure 7: HEPA Filter
Source: World Health Organization (WHO), Laboratory Biosafety Manual 4th Edition

Airflow patterns: Air is drawn into the cabinet through the front opening by the fan motor. Direction, and velocity of airflow in the cabinet varies for different classes of cabinets.

Exhaust system: Exhaust of room air drawn through cabinet may contribute to negative airflow in the culture room. Accessory fan needed to pull air exhausted from cabinet to outside.

TYPE OF BIOSAFETY CABINET

Class I Biosafety Cabinet (Class I BSC)

The Class I BSC provides personnel and environmental protection, but no product protection. Unfiltered room air is drawn in through the front opening (for use by the operator's arms to reach the work surface inside the cabinet), it passes over the work surface and is discharged from the cabinet through the exhaust duct. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and into the exhaust duct. The air from the cabinet is exhausted through an HEPA filter to the outside. (Figure 8)

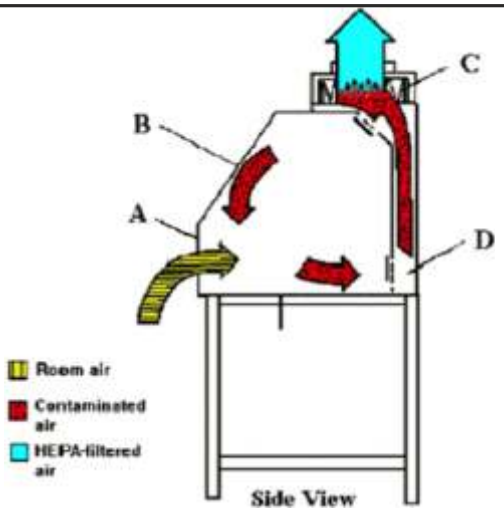


Figure 8: Schematic Representation of A Class I Biological Safety Cabinet. A: Front Opening, B: Sash, C: Exhaust Hepa Filter, D: Exhaust Plenum. (BMBL 5th Edition)
Source: BMBL 5th Edition

Class II Biosafety Cabinet

Class II BSCs differ from Class I BSCs by allowing only air from a HEPA-filtered (sterile) supply to flow over the work surface, therefore providing product protection. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. Airflow is drawn into the front grill of the cabinet, providing personnel protection.

In addition, the downward flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is

particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy or "thimble" connected to the building exhaust.

Class Type II A1 Biosafety Cabinet

- An internal fan (Figure 9) draws sufficient room air through the front grill to maintain a minimum calculated or measured average inflow velocity of at least 75 ft/min at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Airflow provided in this manner reduces turbulence in the work zone and minimizes the potential for cross-contamination.
- The downward moving air "splits" as it approaches the work surface; the fan draws part of the air to the front grill and the remainder to the rear grill. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.
- The air is drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone of the cabinet. Most Class II, Type A1 and A2 cabinets have dampers to modulate this division of airflow.

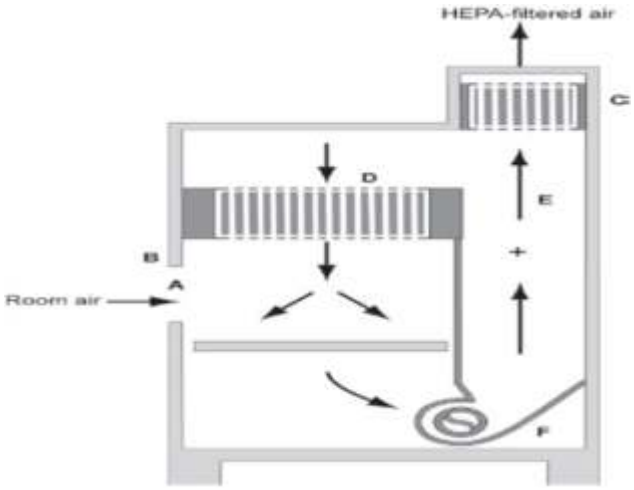


Figure 9: Schematic Representation of A Class II A1 Biological Safety Cabinet. A: Front Opening, B: Sash, C: Exhaust HEPA Filter, D: Supply HEPA Filter, E: Common Plenum, F: Blower (BMBL 5th Edition)
Source: BMBL 5th edition

Class Type II A2 Biosafety Cabinet

A Class II A2 cabinet must have a minimum inflow velocity of 100 ft/min, allowing it to be used for volatile chemicals adjunct to microbiological studies, if properly exhausted outdoors via a canopy exhaust connection. In other respects, the specifications are identical to those of a Type A1.

Exhaust in Type II A1 and Type II A2

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet airflow. The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood, which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing

(Figure 10). The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet.

Note: Class II Type A1 or A2 cabinets should never be hard ducted to the building exhaust system.

Fluctuations in air volume and pressure that are common to all building exhaust systems sometimes make it difficult to match the airflow requirements of the cabinet.

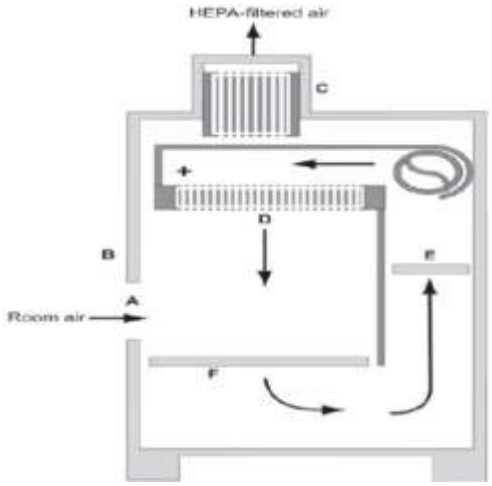


Figure 10: The Tabletop Model Of A Class II, Type A2 BSC (A) Front Opening; (B) Sash; (C) Exhaust HEPA Filter; (D) Supply Hepa Filter; (E) Positive Pressure Common Plenum; (F) Negative Pressure Plenum. Note: The A2 BSC Should be Canopy Connected to the Exhaust
(BMBL 5th Edition)

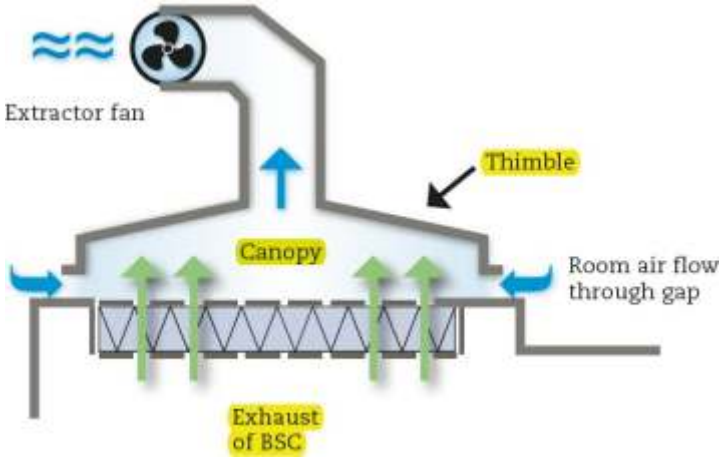


Figure 11: Canopy (Thimble) Unit for Ducting A Class II, Type A BSC (BMBL 5th Edition)
Source: BMBL 5th edition

Table 3: Biosafety cabinet summary

Type	Face velocity (lfpm)	Airflow pattern	Infection precaution level	Product protection
Class I open front	75	In at front, rear and top through HEPA filter	Moderate, High	No
Class II Type A1 and A2	75	70% recirculated through HEPA; exhaust through HEPA, thimble connection	Moderate, High	Yes
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Moderate, High	Yes
Type B2	100	No recirculation. Total exhaust via HEPA and hard ducted	Moderate, High	Yes
Type B3	100	Same as IIA but plenum under negative pressure to room, and exhaust air is ducted	Moderate, High	Yes

BSC: Work practices and procedure Location of Biosafety cabinet:

Ideally, BSCs should be situated in a location remote from traffic and potentially disturbing air currents. Whenever possible a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30–35 cm above the cabinet may be required to provide accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operations

If BSCs are not used properly, their protective benefits may be greatly diminished. Operators need to be careful to maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening.

Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to "air sweep" the surface of the hands and arms. The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. Materials to be placed inside the cabinet should be surface decontaminated with 70% alcohol. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill. Aerosol- generating equipment (e.g. mixers, centrifuges, etc.) should be placed towards the rear of the cabinet. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet. Active work should flow from clean to contaminated areas across the work surface.

The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier and can compromise both personnel and product protection.

Operation and maintenance

Most BSCs are designed to permit operation 24 h/ day, and investigators find that continuous operation helps to control the levels of dust and particulate materials in the laboratory. Class IIA1 and IIA2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use.

All repairs made on BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

Ultraviolet lights

Ultraviolet lights are not required in BSCs. If they are used, they must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure.

Open flames

Open flames should be avoided in the near microbe- free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also used. To sterilize bacteriological loops, microburners or electric "furnaces" are available and are preferable to open flames.

Spills

A copy of the laboratory's protocol for handling spills should be posted, read and understood by everyone who uses the laboratory. When a spill of biohazardous material occurs within a BSC, clean- up should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent

should be disinfected and/or autoclaved.

Certification

The functional operation and integrity of each BSC should be certified to national or international performance standards at the time of installation and regularly thereafter by qualified technicians, according to the manufacturer's instructions.

Evaluation of the effectiveness of cabinet containment should include tests for cabinet integrity, HEPA filter leaks, downflow velocity profile, face velocity, negative pressure/ventilation rate, air-flow smoke pattern, and alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests and it is highly recommended that they are undertaken by a qualified professional.

Cleaning and disinfection

All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth. The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the workday, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms required.

Decontamination

BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination should be performed by a qualified professional.

Personal protective equipment

Personal protective clothing should be worn whenever using a BSC. Laboratory coats are acceptable for work being performed at Biosafety Levels 1 and 2. A solid front, back-closing laboratory gown provides better protection and should be used at Biosafety Levels 3 and 4 (except for suit laboratories). Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator's wrists. Masks and safety glasses may be required for some procedures.

Alarms

BSCs can be equipped with one of two kinds of alarms (Sash and Airflow). Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the operator has moved the sash to an improper position. Corrective action for this type of alarm is returning the sash to the proper position. Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This represents an immediate danger to the operator or product. When an airflow alarm sounds, work should cease immediately, and the laboratory supervisor should be notified. Manufacturers' instruction manuals should provide further details. Training in the use of BSCs should cover this aspect.

References:

1. WHO biosafety Manual, 3rd edition, 2004
2. BIOSAFETY MANUAL FOR PUBLIC HEALTH LABORATORIES, MoHFW, GoI

Spill Management

The purpose of this SOP is to lay down procedures in responding to spills/ accidents while dealing with patient samples, bio medical waste or chemicals while sample processing in the laboratory.

Abbreviations & Definitions

Hazardous material	Hazardous materials are chemicals or infectious biological substances that maypose risks to the safety and health of the staff of----------
Infectious material	Infectious Substances are characterized by their ability to cause infection and disease. E.g. blood, body fluids, bio medical waste
Hazardous chemicals	Hazardous chemicals are chemicals characterized by their flammability, corrosivity, reactivity, or toxicity.
Flammable chemicals	These are chemicals that can burn and/or explode and include liquids with low flash points, flammable solids, flammable compressed gases, and oxidizers. E.g.lighter fluid, aerosol paint, gasoline.
Corrosive materials	These are materials that can eat through containers and combine with other chemicals. They can burn skin and eyes on contact. e.g. caustic soda, hydrochloric acid.
Reactive materials	These are materials that can explode, ignite or produce toxic vapours when exposed to air, water, or other materials. E.g. chlorine, ammonia.
Toxic materials	These are materials that can enter the body through inhalation, ingestion, skin absorption, or injection. These include poisons that may create acute or chronichealth effects (**acute effects** are immediate and short term; **chronic effects** develop over time and are long-term). E.g. Lead, chlorine.
Hazardous material release	Release of hazardous materials via spill, leak, or otherwise into the workplace orthe environment.
Spill	A spill is defined as a hazardous material out of control. The quantity of the biohazardous material spilled is not the sole determining factorin deciding whether an event is classified as a spill. Rather, the essential issue is whether the biological agent, the location, and the quantity collectively cause the situation to be beyond the control of the laboratory worker.
Small/ minor spill (manageable spill)	This is a minor or non-emergency event that presents limited risk to the health andsafety of staff. Such events are typically small in quantity, have little potential for human exposure, and involve materials of low toxicity.
Large/ major spill	This is a significant event and can result in major risks to the health and safety of staff and customers, as well as damage to the environment and property. Such events constitute real or threatened emergency situations, and can involvefire, explosion, or severe biological/ chemical exposure. This may require a coordinated response from an outside organization (e.g., fire department) to safely contain, cleanup, or otherwise respond to the release.
HRG 1	High Risk Group 1 organism Agents those are not associated with disease in healthy adult humans.
HRG 2	High Risk Group 2 Agents that are associated with human disease which is rarely serious and forwhich preventive or therapeutic interventions are often available
HRG 3	High Risk Group 3 Agents those are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk butlow community risk).

Procedure

Biological Spill Clean up Requirement: Spill Kit

Clean up procedure inside BSC

1. Leave the cabinet switched on.
2. Put on gloves and a lab coat.
3. Spray or wipe cabinet walls, work surfaces, and equipment with disinfectant equivalent to 1% hypochlorite solution, (in case of BSC used in TB lab use 5% phenol). If necessary, flood the work surface including drain pans and catch basins below the work surface with disinfectant.
4. Wait for at least 20 minutes.
5. Soak up the disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
6. Discard all clean-up materials into red biohazard waste bags.
7. Remove gloves and thoroughly wash hands.

Spill outside a Biological Safety Cabinet

1. Hold your breath and leave the area immediately.
2. Alert others in the area to evacuate, to prevent the spread of contamination.
3. Notify section in charge.
4. Post a biohazard warning sign near the spill area. Secure the incident area (use barrier tape if necessary) and restrict admission to only those persons cleaning up the spill.
5. Remove any contaminated clothing and put it into a red biohazard waste bag for disposal according to Biological Waste Disposal (SOP Bio medical waste disposal). Wash exposed skin and hands thoroughly.
6. Put on personal protective equipment (lab coat, gloves, mask, eye protection, shoe covers) and assemble clean-up materials.
7. Wait for 30 minutes before re-entering the contaminated area to allow dissipation/settling of aerosols.
8. Cover the spill with paper towels and gently apply disinfectant (1% sodium hypochlorite, in lab use 5% phenol), proceeding from the outer edge of the spill to its center.
9. Wait for at least 30 minutes.
10. Collect all treated materials and discard them in red biohazard waste bags. Use forceps to pick up any broken

Box: Biological Spill kit

1. Red Autoclave Bags
2. Heavy duty bags
3. Forceps for picking glass particles.
4. Dustpan
5. PPE (Gloves, mask, lab coat, shoe cover, safety glasses
6. Filter paper/ old news paper
7. Disinfectants (1% hypochlorite/ 5% Phenol and isopropanol)

Table 4: Acid Spill kit

Item	Quantity
Acid Neutralizer Unit Spill Pacs	1
Absorbent Unit Spill Pacs	3
Plastic Disposal Bags & ties	5
Goggles	1
Pair of Gloves and Nitrile Gloves	1
Sponge	1
Scoops	2
Product Insert (Instruction Sheet)	

Table 5: Alkali Spill kit

Item	Quantity
Alkali Neutralizer Unit Spill Pacs	1
Absorbent Unit Spill Pacs	3
Plastic Disposal Bags & ties	5
Goggles	1
Pair of Gloves and Nitrile Gloves	1
Sponge	1
Scoops	2
Product Insert (Instruction Sheet)	

Table 6: Flammable Solvent Spill kit

Item	Quantity
Flammable Solvent Pure Pacs	1
Absorbent Unit Spill Pacs	3
Plastic Disposal Bags & ties	5
Goggles	1
Pair of Gloves and Nitrile Gloves	1
Sponge	1
Scoops	2
Product Insert (Instruction Sheet)	

glass and discard it in a sharp container.

11. Wipe the spill area again with disinfectant.
12. Remove gloves and PPE, discard in red bag and wash hands thoroughly.

Chemical Spill Cleanup

It is very important for a person handling chemical to know the chemical he/she is handling as the materials required for cleanup will be different.

Clean up Procedure

1. If the spill is in a public area such as a corridor, warn others to leave the area. Notify all individuals in the immediate vicinity of the spill. If necessary, they should move a safe distance away from the spill location. Barricade the spill area with signs to keep away from the hazard.
2. Identify the spilled material, if necessary, by checking labels, shipping papers and/or Material Safety Data Sheets. Identify any immediate hazards, including flammability, toxicity and any surrounding or contributing hazards.
3. For flammable or combustible liquids, eliminate all potential sources of ignition immediately.
4. Each laboratory should have basic spill control materials, as a minimum, spill control pillows, plastic bags and nitrile gloves.
5. Wear appropriate personal protective equipment (protective clothing, goggles or face shield, gloves, footwear [and respirator if indicated]).

Note: Ventilate the spill area if possible. Vapors will be emitted from the spill, regardless of the size. Open doors, and if possible, windows, to an outdoor fresh air environment.

6. Estimate the volume of the spill and place an appropriate number of spill control pillows on the spill or encircle large spills with spill control socks to prevent their spread. Place the pillow on the spill and allow the absorptive action of the spill control pillow to absorb the spill.
7. Use forceps or heavy gloves to pick up any broken glass. Discard the glass into the broken glass bucket.
8. Place pillows in yellow polyethylene bags. Seal and label contents.

Note: Spill control pillows DO NOT contain any chemicals designed to make liquids less toxic, hazardous or flammable. Liquids, when contained in any absorbent material, will continue to be unsafe. Therefore, exercise extreme care when handling, storing, or disposing of spill control pillows containing such liquids.

9. For a spill of liquid acid or alkali, use the appropriate neutralizer to finish the cleanup. See instructions on neutralizer bottle.
10. For a spill of a dry chemical, sweep up the chemical with a dustpan and broom and dispose of into a yellow polyethylene bag. Label
11. For a spill of a dry chemical or after the use of neutralizers, wash the surface with detergent and water and clean by ordinary means.
12. Double bag the yellow polyethylene bag with a second yellow polyethylene bag. Label it and transfer it to the appropriate room of the chemical storage area.
13. Inform the Section in charge. Document the spill.

Mercury Spill Clean up

If possible, replace all mercury containing equipment with non-mercury containing equipment.

Materials required.

1. Latex or rubber gloves
2. Tweezers
3. Goggles
4. Damp paper towels

5. Rubber teats attached to Pasteur pipettes/ squeegee.
6. Plastic dustpan
7. Yellow waste bags
8. Ziploc plastic bags
9. Flashlight
10. Wide-mouth plastic/glass container with screw- on lid
11. Large tray or box
12. Eye dropper or other suction device such as a turkey baster
13. Index cards, playing cards, or other rigid paper to pick up mercury.
14. Sulfur powder
15. Tape – electrical or duct tape works best for small mercury droplets.

Clean up Procedure

1. Alert and keep everyone from the incident room to prevent the spread of contamination.
2. Before sending anyone out of the incident room, check for mercury on clothing and the bottom of shoes. If mercury is visible on any article of clothing or shoes, remove the articles from the person and keep the articles in the incident room.
3. Keep the incident room under 70^0F to minimize mercury evaporation. Close all heating/air conditioning systems that could circulate air from the spill area to other parts of the home or building, till the spill cleans up.
4. Close the inside doors of the incident room. Use a fan in the incident room to move the inside air to the outside. Keep air flowing through the incident room.
5. If any other person has come in contact with the mercury, stay in the area, remove clothing to avoid spread.
6. If you cannot see the mercury, use a flashlight to look for mercury beads. Shine the flashlight at different low angles on the spill area. The light will reflect off the shiny mercury beads to make it easier to see them. For the best results, turn off other lights in the incident room.

If you cannot find the mercury:

7. If the spill is from a fever thermometer, ventilate the room for at least 24 hours moving air through the incident room to move the mercury vapors outside. Make sure to keep doors closed so other areas of the lab are not contaminated with mercury vapors from incident room.
8. Contains the spill, Dike the mercury using rags or other disposable items to prevent spreading. Make sure the mercury does not move to drains, cracks, or crevices or on to sloped or porous surfaces. If you leave the incident room, make sure your clothes and shoes are not contaminated with mercury.

If the mercury was spilled on a hard surface:

9. If the mercury spill involves glass pieces, such as from a glass thermometer or a glass ampoule from a mercury thermostat, use tweezers to safely pick up any broken glass and place it in the plastic bag or container.
10. Work from the outside of the spill area to the center of the spill area. Push the mercury beads together with a card, stiff paper, or squeegee to form larger droplets. Mercury beads roll very quickly, so be careful! Push the mercury beads into a plastic dustpan or use an eyedropper or turkey baster to pick up the beads. If you use an eyedropper, hold it almost parallel with the floor or it will not work very well. You can also use tape to pick up the little beads of mercury but be careful because they might not always stick. Collect all mercury into a leak-tight plastic bag or wide-mouthed sturdy plastic container with a screw on lid.
11. When you think you've picked up all of the mercury, shine a flashlight (at different low angles) on the

area to help find any remaining mercury beads or glass. The light will reflect off the shiny mercury beads and glass.

12. **Optional step:** Sprinkle sulfur powder (if available) on the spill area after cleaning up the beads of mercury; a color change from yellow to brown indicates that mercury is still present, and more cleanup is needed. If the sulfur powder stays yellow, you may stop cleanup efforts.

13. If the mercury was spilled on a porous surface such as hardwood tabletop, the mercury can seep into cracks and crevices. In this case, the mercury cannot be completely removed and, if possible, should be sealed into the surface with epoxy paint, polyurethane or other sealing agent.

If the mercury was spilled on carpet or other cloth material:

14. If the item is removable and disposable (small rug, furniture cover, sheet, etc.) and the mercury beads are still visible, gather up the material carefully with the mercury in the middle of the item and put it in a trash bag. Tightly seal the bag and save for recycling. If the mercury is not visible, you should hang the item outside to air out thoroughly for at least 24 hours.

15. If the item is not removable (carpeting, furniture, etc.), use an eyedropper to collect the visible mercury beads. If possible, use a squeegee or rigid paper such as playing cards or business cards to collect the mercury. You can also use tape to pick up the little beads of mercury but be careful because they do not always stick. The only way to make sure you remove all the mercury is to cut out the area of carpeting, furniture, etc. that had the spill.

16. Use a flashlight to look for mercury beads that you may have missed. The light will reflect off the shiny mercury beads to make it easier to see them.

17. Place the mercury into a leak-tight plastic/ glass container containing water till further action. (if possible, contact vendor to recycle)

Disposing of mercury-containing material

18. Anything that has come in contact with mercury, to be discarded in yellow bag.

19. Inspect your shoes and clothing for mercury. If you find mercury and cannot remove the mercury from these items.

20. Continue to air out the spill room with outside air for 48 hours if weather permits.

21. After you finish your mercury clean up, wash your hands.

22. It is very important that all lab staff be trained to handle hazardous materials only after wearing PPE, so that no material comes in direct contact with body parts. In case of contact with body parts.

23. Wash the affected part with ample water immediately.

24. Stand fully clothed under the safety shower and turn on the shower. Remove wet clothes; wear the lab coats kept in the anteroom.

25. Put wet clothes in red bags and autoclave the street clothes.

26. Inform the lab in charge of all accidents and incidents.

27. When necessary, contact the medicine department for further assistance and use of PPE.

References

1. Laboratory Biosafety manual, WHO 2004

2. Environmentally sound management of mercury waste in health care facilities – CBCB, New Delhi 2010.

GOOD LABORATORY PRACTICES

Human error, poor laboratory techniques and misuse of equipment and not following laboratory safety instructions cause most laboratory injuries and work-related infections. This chapter provides information on the technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

Safety in the laboratory requires every employee's participation and cooperation. Noncompliance with safety precautions not only endangers the individual, but also compromises the health and safety of fellow workers.

Standard Precautions

Standard Precautions represent the minimum infection prevention measures that apply to all patient care, regardless of suspected or confirmed infection status of the patient, in any setting where healthcare is delivered. Standard Precautions replaces earlier guidance relating to Universal Precautions and Body Substance Isolation.

Standard Precautions include: 1) use of personal protective equipment (e.g., gloves, gowns, facemasks), depending on the anticipated exposure, 2) hand hygiene, 3) safe techniques/practices.

Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) may act as a barrier to minimize the risk of exposure. The clothing and equipment selected is dependent on the nature of work performed, type of the pathogen and its transmissibility. PPE should be worn when working in the laboratory. It should be removed, and hands should be washed before leaving the laboratory.

(A.) Gloves

Can reduce the incidents of contamination of hands but cannot prevent penetrating injuries by needles and other sharp instruments.

Gloves should be:

- Well-fitting disposable gloves.
- Heavy-duty general-purpose rubber gloves for washing infected glassware/sharps

Uses of Gloves

- Worn while collecting/handling blood specimens, blood soiled items or whenever there is a possibility of exposure to blood or body fluids containing blood.
- Worn while disposing laboratory waste.

When to change gloves

- Must be changed if visibly contaminated with blood/breached.
- The heavy-duty gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, discolored, or if they have puncture, tears etc.
- Should be removed before handling doorknobs, telephones, pens, performing office work and leaving the laboratory.

(B). Laboratory gowns

- Laboratory gowns prevent contamination of clothing.
- Laboratory gowns or uniforms (preferably wrap-around gowns) should be worn when in the laboratory and should be removed before leaving.
- Front opening lab coats/gowns must be buttoned up while working in the laboratory and must be with full sleeves.
- Plastic aprons should be used while cleaning infected re-usable items and during disposing of waste.

(C). Facial protection

- Facial protection reduces the impact and splash on face/eyes/mouth.
- Simple protective glasses/goggles or face shields may be worn if splashing or spraying of blood/body fluids is expected. These should not be worn outside the laboratory.

(D). Masks

Masks if used correctly may protect the user from aerosol/droplet borne/air-borne infection. The mask to be used is related to the particular risk profile of the category of personnel and his/her work. The risk categorization may change according to the expected degree of environmental contamination and lethality of the agent. Two types of masks are recommended for various categories of personnel depending upon the work environment.

- Triple layer surgical mask
- N 95 Respirator N-95 and triple layer mask is used while handling of patient 's specimens who are suspected of novel influenza viruses.

(E). Occlusive bandage

All skin defects, e.g., Cuts, scratches or other brakes must be covered with waterproof dressing before handling infectious materials.

Hand washing

Hand washing is the single most important means of preventing the spread of infection. Hands should be washed between patient contacts and after contact with blood/ body fluids, secretions, excretions and equipment or articles contaminated by these.

- The role of hands in the transmission of infections has been well demonstrated and can be minimized with appropriate hand hygiene.
- Hands should be washed thoroughly in running water with soap without missing any area.
- Washing of hands is mandatory after
    - o Contamination with blood / body fluids
    - o After removing gowns / coats and gloves
    - o Before eating / drinking and leaving the laboratory

Safe techniques

- No eating, drinking, smoking, applying cosmetics in lab.
- No cell phones used inside the lab.
- No mouth pipetting.
- No placing pencils or pens in the mouth
- Keep your hands away from your eyes and face.
- Always wash hands before leaving lab
- Hand washing
- Handling of sharps: extreme care should be taken.
- Safe technique- follow SOP.

Specimen collection and handling

Improper collection, transport, and handling of specimens in the laboratory not only carry a risk of infection to the personnel involved but also will not be useful for testing/diagnosing infectious organisms.

Ensure sample collection containers/ vials are properly tightened.

- Always grasp the tube or outside of the specimen container, not the stopper or cap, when picking up tubes or specimen containers to prevent spills and breakage.

- Do not keep sample on request form (keep it separate)
- Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes/ zip locks whenever the specimen needs to be transported.
- Containers should be correctly labelled to facilitate identification.

Figure 12: Do not keep Specimen on Request form
Source: BIOSAFETY MANUAL FOR PUBLIC HEALTH LABORATORIES
National Centre for Disease Control
Directorate General of Health Services
Ministry of Health and Family Welfare

Specimen Transport within the Facility

To avoid accidental leakage or spillage, secondary leak proof containers should be used so that the specimen containers remain upright.

- The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.
- The outer container should be rigid and sturdy.

Receipt of specimens in the laboratory

- Laboratories that receive large numbers of specimens should have a designated room or area for this purpose. It is preferable to have a computerized system for record maintenance.
- Leaking specimen containers, requisition forms smeared with specimens, and improperly labeled specimen containers should not be accepted.

Separation of Serum

Always follow standard operating procedure and take care of following:

- o Hands, eye, and mucous membrane protection should be worn. (Standard precaution)
- o Splashes and aerosols can only be avoided or minimized by good laboratory techniques.
- o Blood and serum should be pipetted carefully, not poured.
- o Pipetting by mouth must be forbidden.
- o Pipette tips/disposable pipettes must be disposed in a discarding jar containing suitable disinfectant like 1% sodium hypochlorite.

Films and smears for microscopy

Fixing and staining of blood, sputum and fecal specimens for microscopy do not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately, and decontaminated and/or autoclaved before disposal.

Handling of Lyophilized biological material

Opening vials of freeze-dried (lyophilized) material can be hazardous because these fine dry powders are easily dispersed into the atmosphere when air rushes into the evacuated vessel.

The following procedure may be used to safely open a vial containing lyophilized material.

- Place the ampule/vial and the suggested diluent (water or medium as appropriate) in a BSC.
- Wear gloves, face mask and laboratory coat when opening lyophilized vials.
- Remove the aluminum crimp from the vial. Discard the crimping material into the sharp container.
- Cover the stopper with a moistened gauze pad, and carefully lift the edge of the stopper and allow air to slowly enter the vial. Do not disturb the contents of the vial.
- Once the vacuum has been released, remove the stopper completely and place the stopper upside down on absorbent paper/towel dipped in disinfectant.

- Add the appropriate amount of diluent to the vial using a sterile pipette.
- Replace the stopper and allow the vial contents to hydrate for several minutes.
- Discard the gauze and absorbent paper with other contaminated materials.
- Using a pipette, transfer the contents of the vial to an appropriate container.
- Discard the original vial with other contaminated materials.

References

1. Laboratory Biosafety manual – WHO 2004
2. Environmentally sound management of mercury waste in health care facilities– CBCB, New Delhi 2010.

STERILIZATION AND DISINFECTION

Definitions

Cleaning is a process which removes foreign material (e.g., soil, organic material, microorganisms) from an object.

Disinfection: Chemical means of killing microorganisms, but not necessarily spores.

Disinfectant: A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects. Disinfectants used should be in proper concentration and for a suitable period.

High level disinfection: High-level disinfection traditionally is defined as complete elimination of all microorganisms in or on an instrument, except for small numbers of bacterial spores.

Sterilization: A process that kills and/or removes all classes of microorganisms and spores.

Decontamination of disposable items Used needles and syringes.

- Do not detach needle from syringe.
- Do not recap the needle.
- Cut the used needle in a needle cutter.
- Dispose of the cut needle into a puncture proof, tamper proof sharp container for autoclaving followed by shredding.
- Dispose the syringe in red bag for autoclaving followed by shredding.

Other disposable items Materials required:

- 1% sodium hypochlorite
- Glass jar
- Bio-waste bag (puncture resistant with appropriate color code)
- Gloves

Procedure

- Freshly prepare requisite quantity of disinfectant in a jar for this purpose.
- Label the jar with the name of disinfectant and date of preparation.
- Put articles to be discarded in the solution for minimal contact time/overnight.
- Drain off disinfectant.
- The material for disposal has to be put in appropriate colored biohazard bags & disposed of as per Biomedical waste management rules.

Note: Always prepare fresh solution of disinfectant before use as ready to use solution has shorter shelf life, compared to concentrated one and will be of no use if not freshly prepared. Care should be taken while handling & preparing the solution as it may be corrosive to skin.

Glassware Containing Culture Material.

Materials required

- Puncture proof and autoclavable containers

Procedure

- Discard all the glassware contaminated with culture material directly into puncture resistant and autoclavable containers/metal box. Place box/container with material to be decontaminated in autoclave designated for this work only. Decontaminate the material by autoclaving. Drain off leftover liquid appropriately and pass on material for further washing.

Note: Be sure that decontamination should only be done with autoclaves designated for this purpose. Autoclave should be checked for its efficacy using commercially available chemical indicator strips with every autoclave cycle (show color change indicating appropriate autoclaving) and biological indicator at least once every week.

Table 7: Common Liquid disinfectant

Common Liquid Disinfectants		Usage Requirements		Active Against (positive effect +, no effect -, variable effect *)						
Category	Example	Dilutions	Contact Time (mins)	Vegetative Bacteria	Bacterial Spores	Lipid viruses	Non-lipid Viruses	HIV	HBV	TB
Quaternary Ammonium Compounds	Lysol	1:256	10	+	+	+	-	+	-	-
Phenols	Phenol	1-5%	10-30	+	-	+	*	+	*	+
Chlorine	Chlorox	5-10%	10-30	+	+	+	+	+	+	+

Common Liquid Disinfectants		Usage Requirements		Active Against (positive effect +, no effect -, variable effect *)						
Category	Example	Dilutions	Contact Time (mins)	Vegetative Bacteria	Bacterial Spores	Lipid viruses	Non-lipid Viruses	HIV	HBV	TB
Iodophors		0.5-10%	10-30	+	-	+	+	+	*	+
Ethyl Alcohol		70-85%	10-30	+	-	*	*	+	*	-
Isopropyl Alcohol		70-85%	10-30	+	-	*	*	+	*	-
Formaldehyde		0.2-8%	10-30	+	+	+	+	+	+	+
Glutaraldehyde	Cidex	2%	10-30	+	+	+	+	+	+	+

Washing of laboratory glassware

The type of glassware, i.e., new and dirty/ used is subject to washing for further use. The method used for each type is described below.

New glassware

Purpose

Usually new glassware is slightly alkaline in nature. Before washing, this alkaline nature must be neutralized for final use.

Material required

- 2% hydrochloric acid
- Big plastic basin
- Demineralized water
- Hot air oven for drying purpose only

Procedure

- Prepare sufficient quantity of 2 % hydrochloric acid (e.g., 98 ml of water & 2.0 ml hydrochloric acid) as per the requirement in a big plastic basin.
- Wash the newly received glassware under running tap water to remove the visible dust sticking inside and/or outside surface of the article.
- Soak the already washed articles in 2% hydrochloric acid solution.
- Leave them overnight.
- Take the articles from 2 % hydrochloric acid and rinse in clean water twice.
- Finally, wash using demineralized water. Allow to dry using a hot air oven.
- Pass on for packing & sterilization for further use.

Note

- Care should be taken while using HCl.
- **Add acid to water drop by drop by constant stirring (and not vice versa)**

Dirty glassware

Material required

- 1 % detergent solution.
- Cotton or aluminum foil for plugging.
- Washing brush
- Good quality water supply
- Hot air oven
- Draining rack
- Wire basket for drying
- Demineralized water.

Procedure

- Take material, glassware etc. already decontaminated (chemically/ autoclaving) and rinse twice in lukewarm water to remove any dirty stain sticking on them.
- Put the material to be washed in bowl containing 1% detergent solution.
- Allow it to boil.
- While in solution, scrub inside & outside surface of the glassware with the help of the brush
- Leave the glassware in the solution for 2-3 hrs.
- Take out each article one by one and rinse under running tap water till no trace of detergent is left, which otherwise may lead to false results when used.
- Drain the water by putting each article on a draining rack or by keeping articles upside down in a wire basket.
- Put articles in wire basket and keep in hot air oven at 60°C for drying purpose only.
- Take out each article and plug using non- absorbent cotton/aluminum foil.
- Sterilization of glassware can be done using dry heat or by autoclaving.

Methods of Sterilization

Sterilization is carried out by steam under pressure, dry heat, gas or liquid chemicals. The choice of methods like autoclaving, use of hot air oven etc. Depends on a number of factors including the type of material of the object, number and types of organisms involved and risk of infection to patients or staff. Any sterilization procedure should be monitored routinely by mechanical, chemical, and biological techniques. Sterilized items should be protected against recontamination.

Depending upon the nature of material to be sterilized, sterilization procedures used in microbiology laboratory can be divided into the following categories.

- Dry heat
- Moist heat
- Filtration

Dry heat

The commonly used methods to sterilize the material are as follows.

- Flaming
- Hot air sterilization.

Flaming Purpose

- Used to sterilize material, such as, inoculating wire/ loop, tip of the forceps, searing iron, scalpel etc.

Material required

- Bunsen burner attached to gas supplies.
- Match box.

Procedure

Light the burner with the help of a match box. Adjust the cone of the flame to blue. Hold the inoculum wire/ loop/ tip of the forceps etc. vertically and heat till it gets red hot. Allow it to cool before use. Put off the flame.

Note: Each time when heating in the Bunsen burner flame, allow to cool down the instrument. Check loop/ wire etc. by touching a portion of the medium to be inoculated. Heat the loop vertically so that the entire length of the loop is heated. Dip the loop in disinfectant solution before heating to avoid splattering.

Hot air Sterilization Purpose

The method is used for sterilizing the material like dry glass test tubes, Petri dishes, flasks, glass pipettes, all glass syringes etc. and instruments like forceps, scalpels etc.

Procedure

Arrange the material (prewashed & packed) to be sterilized, loosely and evenly on the racks of the oven so that air can circulate properly and heat the load evenly in the oven. Note the time when desired temperature is reached (Heating time). Hold the load on the same temperature for the specified period as mentioned below.

Temperature Holding Time

- 160 °C for 60 minutes.
- 170 °C for 40 minutes.
- 180 °C for 30 minutes.

The most common temperature for hot air oven for sterilization is 160 °C for 60 min. On completion of the holding time, switch off the power supply and allow the oven to cool down slowly. Open the Hot air oven not before the temperature has come down to 80 °C and take out the sterilized material. Put down the date of sterilization on each packet and store in dust free area for future use. Maintain daily records of the equipment/ material sterilized as per the Performa given below:

Date	Details of item to be sterilized	Temperature at which sterilization was done	Starting time	Time when the set temperature is reached	Time when switched off	Holding time	Chemical indicator tape (color changed)

Precautions:

- Dry up all the material before putting into sterilization in hot air oven.
- Don't place heat sensitive material inside the oven.
- As air is a poor conductor of heat, do not pack the material to be sterilized in the oven too tightly.
- After the holding time is over, the hot air oven is switched off, wait until the temperature of the oven falls below 80°C. Only then open the door of the oven to take out the material otherwise opening immediately after holding time leads to breaking of the glassware and may also cause injuries to the operator.

Moist heat

Moist heat or steam under pressure is one of the most efficient methods of sterilization. Depending upon the material to be sterilized moist heat can be applied in different forms as discussed below.

Autoclave

Autoclaves use pressurized steam to destroy microorganisms and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

Principle

When water boils, its vapor pressure is equal to surrounding atmospheric pressure. When boiling is done in a closed vessel, there is increase in the inside pressure of vessel which raises the temperature of boiling water above 100°C. Saturated steam under pressure is more efficient way of sterilization as compared to dry heat because it provides greater lethal action. It is quicker in heating up the exposed articles. It penetrates porous materials such as cotton wool, stoppers, paper, cloth wrappers etc.

Type of Autoclaves

The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed pre vacuum sterilizer. In the former, steam is admitted at the top or the sides of the sterilizing chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. Gravity displacement autoclaves are primarily used to process laboratory media, water, pharmaceutical products, regulated medical waste, and nonporous articles whose surfaces have direct steam contact. For gravity displacement sterilizers the penetration time into porous items is prolonged because of incomplete air elimination. For example, to decontaminate 4 Kg of microbiological waste requires at least 45 minutes at 121°C because the entrapped air remaining in a load of waste greatly retards steam permeation and heating efficiency. The high-speed pre-vacuum sterilizers are similar to the gravity displacement sterilizers except they are fitted with a vacuum pump (or ejector) to ensure air removal from the sterilizing chamber and load before the steam is admitted. The advantage of using a vacuum pump is that there is nearly instantaneous steam penetration.

Temperature Pressure Time

Recognized minimum exposure periods for sterilization are 30 minutes at 121°C in a gravity displacement sterilizer or 4 minutes at 132°C in a pre-vacuum sterilizer.

Note

- *At the end of holding time switch off the power supply*
- *Allow the autoclave to cool slowly which can be seen by gradual decrease in pressure till it shows zero reading.*
- *Allow the wrapping paper to dry.*
- *Put date on each article and place in dust free area for future use.*
- *Ensure that air from chamber has been expelled completely because air steam mixture has a lower temperature than steam e.g. temperature of 50% air & 50% steam mixture will be 112°C instead of 121°C provided by the pure steam*
- *As the simple autoclave lack means for drying the load after sterilization, it is therefore important to avoid placing sterilized articles in contact with unsterilized objects/ surface unless the wrapping is dried.*
- *To check the efficacy of autoclave, each cycle should be run using chemical indicator tape.*

Quality control

In order for a product to be considered sterilized, it is necessary to verify that all the stages of the sterilization process have been carried out correctly. To verify that these have been fulfilled, various tests have been developed to evaluate the characteristics of the process. The steam cycle is monitored by mechanical, chemical, and biological monitors. Steam sterilizers usually are monitored using a printout (or graphically) by measuring temperature, time and pressure. To monitor effectiveness of the cycle, chemical indicator tapes are used, change of color uniformly indicates that the process has been completed satisfactorily.

Chemical indicators

Most commonly, chemical indicators for steam sterilization/hot air oven are printed inks on packaging materials, or paper strips on which the chemical indicator is printed. A feature of paper strip indicators is that they can be placed inside packs being sterilized and thus checked by the end user. Chemical indicators need to be used for every item in every sterilized load combined with an electronic printout of sterilization parameters for each load. One has to keep in mind that chemical indicators by themselves do not guarantee that the sterilization process complied with all the requirements: personnel who use these must receive precise training to allow them to determine if the result obtained is coherent with the evolution of the whole sterilization process.

Biological indicators

Biological monitoring is the use of living microorganisms for checking and challenging a sterilization process. The goal in using biological indicators is to determine whether all the microorganisms have been killed during the sterilization process. These are considered the best methods for controlling the quality of a sterilization process. The microorganism based biological indicator is a system in which a large number of living hard-to-kill spores of a chosen bacterial species are presented either in a small paper envelope or in a self-contained vial. *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*), a hardy spore, is the organism of choice when monitoring steam sterilization.

Bacterial spores of *B. atrophies* (also called *Bacillus subtilis var niger*) is used with dry heat. The spore indicator is placed in the sterilizing load. The deactivation of spores during the sterilization stage is indicated by their inability to grow in a suitable growth medium over a long incubation time (8 to 72 hours) following the sterilization cycle. These tests are standardized, and manufacturers indicate how to use them and interpret the results.

References

1. Laboratory Biosafety manual, WHO 2004
2. Environmentally sound management of mercury waste in health care facilities–CBCB, New Delhi 2010.

AUTOCLAVE

Moist heat in the form of saturated steam under pressure is the most effective and reliable means of sterilizing glassware, media solutions (when appropriate), and decontamination of biological waste where all forms of microbial life are destroyed.

- The autoclave is a sealing chamber which can generate pressure and heat. At a high pressure, steam becomes saturated, and the temperature is 121°C.
- Three factors are essential for the optimum function of the autoclave.
  - o All air inside the autoclave is replaced by steam (pressure 15 lbs)
  - o The temperature must be 121°C.
  - o Time: 15-20 min for fresh material, 30 min for decontamination of waste
- Autoclaves should be installed away from the main working area as they are noisy, generate heat into the area, and the release of steam may be a hazard.
- The most common autoclave found in laboratories is the gravity displacement type. Saturated steam enters the top of the chamber by a steam pressure control valve. As the steam enters, it pushes the air out through a trap in the drain line. Once all the air is evacuated, the trap closes. Steam continues to fill the autoclave chamber until a preset temperature and pressure is reached.

Figure 13: Vertical Autoclave
Source: https://www.bionicsscientific.com/autoclave-steril- izer/vertical-type-autoclave-steam-sterilizer.html

Autoclave operating procedure General maintenance

- All operators must complete training on autoclave operation.
- Before operating the autoclave please take a few minutes to read through the operator's manual.

Material Preparation

- Ensure that the material is suitable for autoclave (no chlorination article)
- Prepare and package material suitably.
- Cover all containers with a loosened lid or steam-penetrable bung.
- Wrap / bag loose dry materials in steam- penetrable paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing efficiency of the process.
- Ensure that liquids are not more than 2/3rd the capacity of containers and that the lids are loosened.
- Ensure that plastics are of heat-resistant autoclavable.
- Place Items in a stainless steel or autoclavable plastic container for their stability and ease of handling
- Place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave. The pan must be large enough to contain a total spill of the contents.

Loading autoclave

- Wear heat-insulating gloves, aprons and closed toed shoes.
- Place material in autoclave. Do not mix incompatible materials.
- Do not overload. Leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.
- Close and latch the door firmly.

Operating Autoclave

- Ensure that the water reservoir is filled to BOTTOM of safety value. If not, add water, being sure not to immerse the safety valve.

- Plug the power cord in and turn the main switch to START.
- Fill the autoclave with water by turning the multi- purpose value (always turn this valve clockwise) to FILL. The water should reach most of the width of the "indicator channel" at the front of the chamber. Turn the multi-purpose valve to stop the flow of water and prepare the unit for sterilization.
- Close the door and lock it by turning the handle clockwise. Ensure that the door is tightly closed.
- Set the temperature at 250 °F / 121 °C with the thermostat knob. This is the temperature most often used for sterilization. Set the timer to the correct sterilization period.
- Check on the unit after about 25 minutes to be sure that it is reaching temperature / pressure. Small bursts of steam may be evident from the water reservoir during the run; this is normal and necessary. Record the unit pressure on the Autoclave Use log. It is necessary to record the maximum pressure while the unit is running.
- If the unit does not reach the set temperature, contact Lab supervisor to arrange for professional servicing of the unit. DO NOT USE until the unit is repaired.
- When the timer reaches 0, the run is complete.
- Turn the switch off.
- Record on instrument log sheet

Unloading Autoclave

- Wear heat-insulating gloves, aprons and closed toed shoes.
- Ensure that the pressure of the chamber is zero before opening the door.
- Stand back from the door as a precaution, carefully crack door open no more than 1 inch (2.5 cm) to release residual steam and allow pressure within liquids and containers to normalize.
- Allow sterilized material to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
- After removal from the autoclave, place hot items in an area which clearly indicates the items are 'hot' until the items cool to room temperature.
- Push door closed.

Autoclave maintenance

- Check door gaskets for cracks and pitting.
- Repair door clamps that are difficult to close properly.
- Check pressure and temperature achieved during a cycle- see printout generated by the autoclave.
- Sign and date all printouts and file in logbook
- Repairs and service by qualified personnel
- Any function problems are filed in the logbook along with the corrective action taken to correct the problem.

Autoclave Quality control Chemical Strip test:

- These are typical chemical tests changing color or state when exposed to the different phases of the sterilization process. Chemical indicators allow the differentiation of articles submitted or exposed to a successful sterilization process from those that have not. Among the best known are the adhesive tapes or strips that go inside a component or on packages. Stick the adhesive indicator strip on all items if possible or at least in the centre most article.

Monthly spore testing

- Once per month a biological indicator with a heat resistant isolate of Bacillus stearothermophilus is autoclaved and incubated for growth in enriched media. Results are documented for the effectiveness

of the autoclave.

- Positive growth on a vial that was autoclaved- check the printout if available, autoclave another vial, if still positive, call for service and do not use.

HOT AIR OVEN

Hot air ovens are electrical devices used for sterilizing or drying glassware and metal materials used for examinations or tests performed in the laboratory.

- Dry heat sterilization of clean material is conducted at 160 °C for one hour in the oven. Upon being heated by high temperature dry air, humidity is evaporated from glassware and thus the possibility of any remaining biological activity is eliminated.
- Safe Material Handling: It is very important not to put materials in these ovens that might damage the ovens or create a hazardous situation. Before you put anything in the oven make sure it is safe to heat to the desired temperature.
- Chemical: Oxidation, melting, violent reactions, out-gassing of your specimen or part may create chemical hazards such as toxic, or corrosive, or flammable gasses or liquids. Reactions with the materials used in the inner walls of the furnace might also occur. Think carefully about what you will be placing in these ovens so that potential chemical hazards do not become real chemical emergencies.
- Be careful about electrical, fire, combustion, melting, thermal expansion and fumes while putting anything in Oven.

Figure 14: Hot Air Oven
Source: https://www.amazon.in/FIRST-Litres-Alumini- um-Chamber-12x12x12 /dp/B09SWKB9M5

General operating procedures- Hot air Oven

- Before using the furnace, inspect and ensure electric power connections are made correctly, atleast6 inch clearance of instrument from the wall, make sure it is clean and that no one has left their specimen or any other fixtures in it.
- Wear the necessary PPE.
- Arrange all articles in tray with enough space in between for circulation of air. Ensure that all glass articles are completely dry.
- Make sure articles are not touching the heating elements or an electrical short might occur when you close the door.
- Close the Oven door and turn on the oven by pressing the main power switch.
- Set the knob to desired temperature wait for the oven to reach the desired temperature.
- When desired temperature has reached (as indicated by the thermometer reading) start timer
- Post a tag out indicating the articles kept in & the time the oven has to be opened.
- Remove the PPE and keep it safe.

Removing samples from oven

- Before you start to take anything out of the oven make sure you have everything you are going to need (PPE), especially if the specimen is still hot.
- Make sure there is a clear and safe place to put your sample, also ensure that you have the necessary plan in place in case of any accident.
- Once you have made these preparations use the following procedure to remove your article
- Open the oven door.
- Remove your article, if still hot, carefully place it on a heat resistant surface.

- Close the door.

Quality Assurance/Quality Control of Hot air oven

- Quality control of drying ovens is slightly demanding since sterilization by dry heat has temperature and time as critical parameters. Generally, spores of Bacillus subtilis are used as biological indicators.
- These must be incubated for several hours after the sterilization process.
- Monthly Spore Testing: Each month the spore strips (B subtilis) should be placed in oven. These strips have active spores on them. The strips are placed into the oven and a normal sterilization run completed. The strips are then analyze in the bacteriology section.
- The strips must exhibit no growth; this provides assurance that the hot air oven is efficiently sterilizing materials.

BIOMEDICAL WASTE MANAGEMENT

Biomedical waste management refers to the proper handling, segregation, storage, transportation, treatment, and disposal of waste generated in healthcare facilities. It involves specific protocols and guidelines to ensure the safe management of potentially infectious or hazardous waste.

The key aspects of biomedical waste management:

1. Segregation

Waste should be segregated at the point of generation into different categories based on its nature and potential risk. Common categories include sharps (needles, broken glass, etc.), pathological waste (tissues, organs, body fluids), infectious waste (cultures, swabs, laboratory waste), pharmaceutical waste, chemical waste, and general non-hazardous waste. Each category requires specific handling and disposal procedures. Biomedical waste must be segregated at point of generation.

2. Collection and Storage

Different types of biomedical waste should be collected separately using color-coded bins or containers. The containers should be leak-proof, puncture-resistant, and labeled appropriately. They should be kept in designated storage areas to prevent mixing and unauthorized access. As per BMWM Rules, 2016 and HCF should also ensure disposal of human anatomical waste, animal anatomical waste, soiled waste and biotechnology waste within 48 hours.

- Interim storage of bio medical waste is discouraged in the wards / different departments of HCF.
- If waste is needed to be stored on interim basis in the departments, it must be stored in the dirty utility/sections.
- No waste should be stored in patient care area and procedures areas such as Operation Theatre. All infectious waste should be immediately removed from such areas.
- In absence of dirty utilities/ sections such BMW must be stored in designated place away from patient and visitor traffic or low traffic area.

3. Labelling

All the bags/ containers/ bins used for collection and storage of bio-medical waste, must be labelled with the Symbol of Biohazard or Cytotoxic Hazard as the case may be as per the type of waste in accordance with the BMWM Rules, 2016.

4. Transportation

Biomedical waste should be transported from the healthcare facility to the treatment or disposal facility using dedicated vehicles that comply with safety regulations. The vehicles should be equipped with containment systems to prevent spillage or leakage during transportation. Color coded trollies may be used for transportation of biomedical waste.

5. Treatment

Biomedical waste requires proper treatment to inactivate or destroy pathogens and reduce its potential harm

to public health and the environment. Common treatment methods include autoclaving (steam sterilization), incineration, microwave treatment, chemical disinfection, or alternative technologies such as plasma gasification. The choice of treatment method depends on the type of waste and local regulations.

6. Disposal

After treatment, the waste can be safely disposed of following applicable regulations. Disposal methods may include landfilling for non-hazardous waste, specialized landfilling for treated hazardous waste, or appropriate recycling methods for certain types of waste such as glass or plastics.

7. Training and Awareness

Healthcare workers involved in waste management should receive proper training on the segregation, handling, and disposal of biomedical waste. They should be educated about the potential risks associated with improper waste management and the importance of following protocols to protect themselves, patients, and the environment.

8. Regulatory Compliance

Biomedical waste management is subject to local regulations, guidelines, and permits. Healthcare facilities must comply with these regulations and maintain appropriate records of waste generation, handling, treatment, and disposal.

Proper biomedical waste management is essential to protect the health and safety of healthcare workers, patients, waste handlers, and the community. It helps prevent the spread of infections, reduces environmental pollution, and ensures compliance with regulatory requirements.

Biomedical waste management rule 2016 applies to:

All persons who generate, collect, receive, store, transport, treat, dispose, or handle bio medical waste in any form including hospitals, nursing homes, clinics, dispensaries, veterinary institutions, animal houses, pathological laboratories, blood banks, Ayush hospitals, clinical establishments, research or educational institutions, health camps, medical or surgical camps, vaccination camps, blood donation camps, first aid rooms of schools, forensic laboratories and research labs.

Box 1: Biomedical rule 2016 NOT apply to:
· Radioactive wastes as covered under the provisions of the Atomic Energy Act, 1962(33 of 1962)
· Hazardous chemicals covered under the Manufacture, Storage and Import of Hazardous ChemicalsRules, 1989 made under the Act
· Solid wastes covered under the Municipal Solid Waste (Management and Handling) Rules, 2000made under the Act · **Amendment 2018:** Solid Waste Management Rules, 2016 replaced in place of rule 2000
· The lead acid batteries covered under the Batteries (Management and Handling) Rules, 2001 madeunder the Act
· hazardous wastes covered under the Hazardous Wastes (Management, Handling and Transboundary Movement) Rules, 2008 made under the Act · **Amendment 2018**: Management and Transboundary Movement) Rules, 2016 in place of Rule 2008
· Waste covered under the e-Waste (Management and Handling) Rules, 2011 made under the Act; · **Amendment 2018**: E-Waste (Management) Rules, 2016 in place of Rule 2011
· Hazardous micro-organisms, genetically engineered micro-organisms and cells covered under the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, Genetically Engineered Microorganisms or Cells Rules, 1989 made under the Act

Table 15: Schedule I (Part 1): Biomedical wastes categories, their segregation and collection

Category	Type of Waste	Type of Bag of Container to be used
Yellow	**(a) Human Anatomical Waste**: Human tissues, organs, body parts and fetus below the viability period (as per the Medical Termination of Pregnancy Act 1971, amendedfrom time to time).	Yellow colored non-chlorinated plastic bags  
	(b) Animal Anatomical Waste: Experimental animal carcasses,body parts, organs, tissues, including the waste generatedfrom animalsused in experimentsor testing in veterinaryhospitalsor collegesor animalhouses.	
	(c) Soiled Waste: Items contaminated with blood, body fluids like dressings, plaster casts, cotton swabs andbags containing residual or discarded blood and blood components.	
	(d) Expired or Discarded Medicines: Pharmaceutical waste like antibiotics, cytotoxicdrugs including all items contaminated with cytotoxic drugs along with glass or plastic. ampoules, vials etc	Yellow colored non-chlorinated plasticbags
	(e) Chemical Waste: Chemicals used in production of biological andused or discarded disinfectants.	Separate collection system leading to effluent treatment system
	(g) Discarded linen, mattresses, bedding contaminated with blood or body fluid. Amendment 2018: after the words "body fluid", the words ", routine mask and gown" shall be inserted;	Non-chlorinated yellow plastic bags or suitable packing material
	(h) Microbiology, Biotechnology and other clinical laboratory waste: Blood bags, Laboratory cultures, stocks or specimens of microorganisms, live or attenuated vaccines, human and animal cell cultures used in research, industrial laboratories, production of biological, residual toxins, dishes and devices used for cultures.	Autoclave safe plastic bags or containers **Amendment 2018: "Autoclave or Microwave or Hydroclave safe plastic bags or containers"; may be substituted**
Category	**Type of Waste**	**Type of Bag of Container to be used**
Red	**Contaminated Waste (Recyclable)** (a) Wastes generated from disposable items such as tubing, bottles, intravenous tubes andsets, catheters, urine bags, syringes (without needles and fixed needle syringes) and vacutainers with their needles cut) and gloves.	Red colored non-chlorinated plastic bags or containers 

White (Translucent)	**Waste sharps including Metals:** Needles, syringes with fixed needles, needlesfrom needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded andcontaminated metal sharps	Puncture proof, Leak-proof, tamper-proof containers 
	(a) Glassware: Broken or discarded and contaminated glass including medicine vials and ampoules exceptthose contaminated with cytotoxic wastes	Cardboard boxes. With blue colored marking Amendment 2018 following item shall be substituted, namely: – "(a) Puncture proof and leakproof boxes or containers with blue colored marking 
	(b) Metallic Body Implants Cardboard boxes with blue colored marking Amendment 2018 following item shall be substituted, namely:– "(b) Puncture proof and leak proof boxes or containers with blue colored marking";	

General Waste

General waste consists of all the waste other than bio-medical waste and which has not been in contact with any hazardous or infectious, chemical or biological secretions and does not includes any waste sharps. This waste consists of mainly:

1. Newspaper, paper and card boxes (dry waste)
2. Plastic water bottles (dry waste)
3. Aluminium cans of soft drinks (dry waste)
4. Packaging materials (dry waste)
5. Food Containers after emptying residual food (dry waste)
6. Organic / Bio-degradable waste - mostly food waste (wet waste)
7. Construction and Demolition waste

This quantity of such waste is around 85 % to 90 % of total waste generated from the facility. Such waste is required to be handled as per Solid Waste Management Rules, 2016 and Construction & Demolition Waste Management Rules, 2016, as applicable.

Other waste

Other wastes consist of used electronic wastes, used batteries, and radio-active wastes which are not covered under biomedical wastes but have to be disposed as and when such wastes are generated as per the provisions laid down under E-Waste (Management) Rules, 2016, Batteries (Management & Handling) Rules, 2001, and Rules/guidelines under Atomic Energy Act, 1962 respectively.

Biomedical waste treatment: As per the biomedical waste management rule 2016 (amendments in 2018 and 2019), segregated biomedical waste must need to treated as per table given bellow.

Table 16: BMW treatment/ disposal methods

Category	Type of Waste	Treatment and Disposal options
Yellow	**(a) Human Anatomical Waste** **(b) Animal Anatomical Waste**	Incineration or Plasma Pyrolysis or deep burial*
	(c) Soiled Waste:	Incineration or Plasma Pyrolysis or deep burial* In absence of above facilities, autoclaving or micro-waving/ hydroclaving followed by shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent for energy recovery.
	(d) Expired or Discarded Medicines:	Expired `cytotoxic drugs and items contaminated with cytotoxic drugs to be returned back to the manufacturer or supplier for incineration at temperature >1200 C or to common bio- medical waste treatment facility or hazardous waste treatment, storage and disposal facility for incineration at >1200C Or Encapsulation or Plasma Pyrolysis at >12000C. All other discarded medicines shall be either sent back to manufacturer or disposed by incineration.
	(e) Chemical Waste:	After resource recovery, the chemical liquid waste shall be pre-treated before mixing with other wastewater. The combined discharge shall conform to the discharge norms given in Schedule III.
	(g) Discarded linen, mattresses, bedding contaminated with blood or body fluid.	Non- chlorinated chemical disinfection followed by incineration or Plasma Pyrolysis or for energy recovery. In absence of above facilities, shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent for energy recovery or incineration or Plasma Pyrolysis.
	(h) Microbiology, Biotechnology and other clinical laboratory waste:	Pre-treat to sterilize with nonchlorinated chemicals on-site as per National AIDS Control Organization or World Health Organization guidelines thereafter for Incineration. **Amendment 2018:** for the portion beginning with "as per National AIDS Control Organisation", and ending with "for incineration", the following shall be substituted, namely: – "as **per World Health Organisation guidelines on Safe management of wastes from health care activities and WHO Blue Book, 2014** and thereafter sent for incineration";
Red	Contaminated Waste (Recyclable)	Autoclaving or micro-waving/ hydroclaving followed by shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent to registered or authorized recyclers or for energy recovery or plastics to diesel or fuel oil or for road making, whichever is possible. Plastic waste should not be sent to landfill sites
White (Translucent)	Waste sharps including Metals:	Autoclaving or Dry Heat Sterilization followed by shredding or mutilation or encapsulation in metal container or cement concrete; combination of shredding cum autoclaving; and sent for final disposal to iron foundries (having consent to operate from the State Pollution Control Boards or Pollution Control Committees) or sanitary landfill or designated concrete waste sharp pit.
Blue	(a) Glassware: (b) Metallic Body Implants	Disinfection (by soaking the washed glass waste after cleaning with detergent and Sodium Hypochlorite treatment) or through autoclaving or microwaving or hydroclaving and then sent for recycling.

Reference: https://dhr.gov.in/sites/default/files/Bio-medical_Waste_Management_Rules_2016.pdf

Specimen Collection

Blood Collection

- Blood should be collected by clinical staff of phlebotomist. In some cases, collection of venous blood may be undertaken in the laboratory by or under the direction of a doctor.
- The laboratory director instructs the staff to follow correct procedure as below:
  - i. Identify the patient.
  - ii. Fill out test request form (s) and ensure that the correct identification is marked on the specimen tube.
  - iii. Prepare the patient and cleanse the venipuncture site with 70% alcohol or any other antiseptic solution followed by drying with a clean piece of cotton wool or gauze.
  - iv. Avoid prolonged compression of the vein by torniquet prior to collecting the blood, this should not be more than 1 min.
  - v. Correct anticoagulant for various test
  - vi. Ensure adequate mixing of blood with anticoagulant but avoid frothing by excessive shaking of the sample.
  - vii. Ensure all the tubes are securely capped and placed in an appropriate rack or for sample packaging.
  - viii. Ensure adequate arrangements are made for rapid dispatch/ transport of specimen.
  - **ix. Order of sample collection**: collect blood sample in blood culture tube first, then sod citrate, then serum tubes (clot activators, heparin tube with or without gel, then EDTA tubes with or without get separator, then Sodium fluoride/potassium oxalate glycolytic inhibitor in the last.

Types of test tubes

Different types of sample tubes with different containing additives and/or anticoagulants for various laboratory procedures should be available in the lab.

Table 8: Types of vacutainers for collection of blood samples

	Vacutainer color Cap	Additives	Sample type	Inversion
	Light Blue	Sod Citrate	Whole blood/ plasma	3-4 times
	Black	Sod Citrate ESR	Whole blood/ plasma	8-10 times
	Red	No Additive	Serum	5-6 times
	Gold/ Yellow	SST	Serum	5-6 times
	Green	Lithium Heparin	Plasma	8-10 times
	Purple/ Levender	EDTA	Whole blood	8- 10 times
	Pink		Cross match	
	Grey	Fluoride/Oxalate		8-10 times
	Royal Blue		Trace element	

Anticoagulants

It refers to any substance that inhibits blood coagulation. The choice of anticoagulant depends on the type of examination to be carried out; thus, they are of many types, which include:

I. EDTA (ethylene diamine tetra acetic acid): Dipotassium and disodium salts of EDTA prevent the coagulation of blood by combining with calcium. It has the advantage of preserving the stain ability and morphologic characteristics of leukocytes. EDTA can be used in 2 forms, which are liquid and dry

(powder) form.

II. **Heparin:** It prevents coagulation of blood by interfering with the conversion of prothrombin to thrombin. A 5 mL syringe can be rinsed with 1% heparin solution for highest anticoagulation activity. It has the disadvantage of adversely affecting the leukocyte stain ability.

III. **Sodium Citrate**: It is not commonly adopted for the preservation of blood for hematologic determination and can be used for blood transfusion.

IV. **Oxalates (Sodium and Potassium):** They also prevent coagulation of the blood by combining with calcium. They should not be used as an anticoagulant when blood non protein nitrogen and blood urea tests are required.

Types of Needles

The size of the needle should be 19-21 gauges in adults and 21-23 gauges in pediatrics to avoid hemolysis during sample drawing from the patient and also during evacuation into the test tubes.

Disposables

They include syringe, needle, gauze, swab, gloves, plasters, and lancets.

Consumables

They include various types of disinfectants and sterilizers such as 5% savlon solution, tincture iodine, or 70% alcohol.

Miscellaneous

They include lab coat, gown, apron, permanent marker, vacate and tourniquets.

Figure 15: Types Of Needle (Google Image)
Source: ModifiedPrinciplesofHaematology LabManu- al-Fall2016%20(1).pdf

Sample collection Procedure

Capillary Blood Collection

Blood is collected from capillary network if few drops of blood are required such as measurements of Hb level, RBC count by microdilution method and preparation of thin blood film. Capillary blood cannot be used for platelet testing. The correct procedure of capillary blood collection is given in figure 16. Major sites for capillary blood collection are:

a. Finger prick
b. Ear lobe
c. Sides of the heel especially in pediatric and neonatal patients.

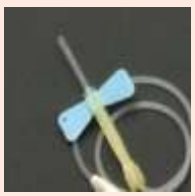
Venous Blood collection: Blood is collected from the venous system of the patient when a large amount of blood is required (follow the steps given in figure 17). Choose veins that are large and accessible and avoid bruised or scarred areas.

Major venous blood sampling sites are:

a. Median cubital veins.
b. Cephalic (dorsal hand) veins.
c. Basilic veins.
d. Great Saphenous (foot) veins.

1	2	3	4	5
				
Ask person to rub/ massage hands together. Ensure the clients hands are warm to touch. Note: Technician should wash hand his/her before	Position hand palm-side up. Choose least calloused finger (either middle or ring finger).	Apply intermittent pressure. to help the blood flow.	Disinfect fingertip. Start in the middle and work outward to avoid contamination. Allow to dry.	Hold the finger and firmly place a new sterile lancet off-center on the fingertip.
6	**7**	**8**	**9**	**10**
				
Firmly press the lancet to puncture the fingertip.	Wipe away the 1st drop of blood with a sterile gauze pad or cotton ball.	Use the loop provided in the kit to collect the blood.	Apply a gauze pad or cotton ball to the puncture site until the bleeding stops.	Properly dispose of all contaminated supplies.

Figure 16: Capillary Blood Collection Procedure

1	2	3	4	5	6
					
Collect supplies.	Wash hands thoroughly and put on a new pair of gloves.	Label tube with the patient identification number.	Put tourniquet on patient about 3-4" above venipuncture site.	Have client form a fist so veins are more prominent.	After palpating the path of the vein, clean the venipuncture site w/alcohol using a circular motion. Allow the area to dry.
7	**8**	**9**	**10**	**11**	**12**
					
Remove needle sheath from butterfly winged infusion set.	Assemble needle and vacuum tube holder.	Use your thumb to pull skintight, 1-2" below venipuncture site.	Insert the needle, bevel side up into the vein. A "flash" of blood will enter the tubing.	Push the vacutainer tube completely into the needle. Blood should begin to flow into the tube.	Have participant relax the fist and release the tourniquet.

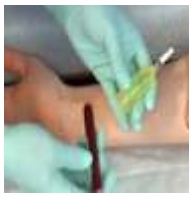
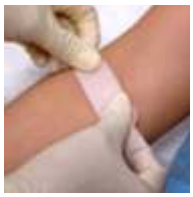
13	14	15	16	17	18
					
Gently invert it 8-10 times before laying tube down to prevent the blood from clotting.	After completion of blood collection, place dry gauze over the venipuncture site.	Remove the needle and apply pressure to the pad until bleeding stops.	Once bleeding stops, apply a plaster.	Properly dispose of all contaminated supplies.	

Figure 17: Venous Blood Collection Procedure

General Cautions/Procedure of venous blood sampling

1. Be quiet with the patient and let him/her feel comfortable.
2. Verify that computer printed labels match requisitions. Check patient identification band against labels and requisition forms. Ask the patient for his /her full name, address, identification number, and date of birth.
3. If a fasting specimen or dietary restriction is required, confirm patient has fasted or eliminated food from the diet as ordered by physician.
4. Position the patient on a chair or a bed properly.
5. Choose the appropriate tube for collection.
6. Apply a tourniquet 3-5 inches above the antecubital fossa for not more than 1 minute.
7. Ask the patient to make (clench) a fist without vigorous hand pumping. Select a suitable site for venipuncture.
8. Feel or palpate for any vein to determine its potential size, depth and direction that may be hidden. If still the vein is not visible or palpable, ask the patient to "pump" the hand 3 times and no more, as it may cause hemoconcentration. If all measures fail to palpate a suitable vein, ask for the opinion from another experienced technician.
9. Put on gloves with consideration of latex allergy for the patient.
10. Cleanse the venipuncture site with 70%isopropyl alcohol. Allow the area to dry.
11. Anchor the vein firmly.
12. Enter the skin with the needle at approximately 30-degree angle or less to the arm with the level of the needle up;
    a. Follow the geography of the vein with the needle.
    b. Insert the needle smoothly and fairly rapidly to minimize the patient discomfort.
    c. If using a syringe, pull back on the barrel with a slow, even tension as blood flows into the syringe. Do not pull back too quickly to avoid hemolysis or collapsing the vein.

If using an evacuated system, as soon as the needle is in the vein, ease the tube forward in the holder as far as it will go, firmly securing the needle holder in place. When the tube has filled, remove it by gasping the end of the tube and pulling gently to withdraw and gently invert tubes containing additive and/or anticoagulant.

Sample Collection for Blood Culture

1) Blood samples for culture should be drawn under aseptic conditions.
2) Clean the skin with savlon, apply 2% Iodine, or 10% povidone wait for a minute and draw the blood. Clean the iodine with alcohol or spirit.

3) Draw out 5 ml of blood for one blood culture bottle containing 50 ml of BHI broth (For pediatric age group, 1 ml in 10 ml of BHI broth in MaCartney's bottles may be added, if available. Otherwise, the same inoculum may be added to 50 ml medium also).

4) Transport to the laboratory if not possible keep in incubator. If not available keep at room temperature NEVER REFRIGERATE.

Figure 18: Mc Macartney Bottle (Google Image)
Source: https://www.labmaterials.net/en/415-mcb1-flacon-en-verre-avec-bouchon-aluminium.html

Urine collection

Mid-stream urine sample is collected after giving proper instructions to the patient (clean the genitalia properly, collect a "clean-catch" mid-stream urine sample in a sterile bottle. Do not touch the inner side of the cap or bottle)

1. Transport immediately to the lab. If more than half an hour delay in expected, refrigerate at 4C. It should be processed within 3-4 hours of collection.
2. Catheterized patients - Do not collect from collection bag or after opening the closed drainage. Clean an area over the collecting tubes and puncture with the help of a sterile needle and syringe and draw out the sample.
3. Suprapubic aspiration under aseptic techniques may be done in infants and where mid-stream results are doubtful.

Figure 19: Urine Container (Google Image)
Source: https://images.app.goo.gl/Rpyn8kDnUu1N9qZm6

Stool Sample Collection

Collect the stool in a dry, clean, leakproof container. Make sure no urine, water, soil or other material gets in the container.

1. The image given below demonstrates the distribution of protozoa in relation to stool consistency and should be taken into consideration when specimens are received.
2. Fresh stool should be examined, processed, or preserved immediately. An exception is specimens kept under refrigeration when preservatives are not available; these specimens are suitable for antigen testing only.
3. Preserve the specimen as soon as possible. If using a commercial collection kit, follow the kit's instructions. If kits are not available, the specimen should be divided and stored in two different preservatives, 10% formalin and PVA (polyvinyl alcohol), using suitable containers. Add one volume of the stool specimen to three volumes of the preservative.
4. Ensure that the specimen is mixed well with the preservative. The formed stool needs to be well broken up.
5. Ensure that the specimen containers are sealed well. Reinforce with parafilm or other suitable material. Insert the container in a plastic bag.
6. Certain drugs and compounds will render the stool specimens unsatisfactory for examination. The specimens should be collected before these substances are administered, or collection must be delayed until after the effects have passed. Such substances include antacids, kaolin, mineral oil and other oily materials, non-absorbable antidiarrheal preparations, barium or bismuth (7-10 days needed for clearance of effects), antimicrobial agents (2-3 weeks), and gallbladder dyes (3 weeks).
7. Specimen collection may need to be repeated if the first examination is negative. If possible, three specimens passed at intervals of 2-3 days should be examined.

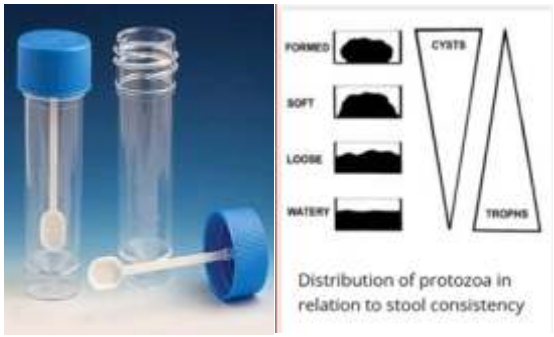


Figure 20: Stool Container (Google Image)
Source: https://images.app.goo.gl/6XEW1jvn6hafdP579

8. Sample collected in Formalin can interfere with PCR, especially after extended fixation time, Inadequate preservation of morphology of protozoan trophozoites, not suitable for some permanent smears stained with trichrome.
9. Sample collected in PVA, Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia, cannot be used with immunoassay kits, Not suitable for acid-fast, safranin and chromotrope stains.

Sputum Collection

Take 50 ml screw capped and graduated conical bottom polypropylene tube. Do not open until ready to use.

1. The mouth should be free of foreign matter and may be rinsed with clean/ bottled water prior to collection.
2. Take deep breaths through your mouth and cough up the mucous from deep in your lungs (Breathing deeply over a pan of boiling water may help raise sputum).
3. Open the container and hold it close to your mouth. Cough the mucous into the container.
4. Collect 2-5 ml specimen.
5. Once collected, screw the lid on tightly
6. Write patient name and other identifier on tube.
7. Store specimen in the refrigerator until transported.
8. Notify Department of Health staff to arrange for transport of specimen to the laboratory.

Figure 21: Sputum Collection Procedure) Https:// Doh.sd.gov/Documents/Diseases/ Infectious/ Sputumcollection.pdf)

Other Sample Collection

CSF/OTHER NORMALY STERILE BODY FLUIDS

1. Collect under aseptic condition and transported immediately to the laboratory.
2. They should not be refrigerated if delay in transport is expected. Keep at room temperature.

PUS, OTHER DISCHARGE, AND OTHER SWABS

1. Do not apply antiseptics before collection. Clean with Normal saline
2. In case of discharge, 1-2 ml of sample in a sterile penicillin vial is preferable to swab.
3. If swabs are sent, 2 swabs in sterile containers should be sent, one for direct examination and one for culture.
4. Vaginal swabs should be high vaginal swabs preferably collected after actual visualization; It should not touch the sides of the vaginal wall.
5. Throat swab (2 in numbers) should be collected under direct visualization without touching the tongue or buccal mucosa. In cases of suspected diphtheria Mention clearly at the right. Mention clearly in the right corner of the form.

CATHETERS AND TIPS:

Mark the junction of Skin and Catheter, withdraw the catheter a little and cut it at about 1 cm distal to the mark and send 5 cm length of the catheter in sterile in container. The transcutaneous part is a better sample than the tip in case of long catheters.

References

1. https://www.cdc.gov/dpdx/diagnosticprocedures/stool/specimencoll.html.
2. GLI Practical Guide to TB Laboratory Strengthening.https://stoptb.org/wg/gli/assets/documents/GLI_practical_guide.pdf
3. SEA/HLM/320, guidelines on standard operating procedures on Hematology, WHO.

SAMPLE STORAGE, PACKAGING AND TRANSPORTATION

- Specimens should ideally be sent to the laboratory as soon as possible (via the next transport on the day of sample collection).
- If samples cannot be sent to the laboratory on the day of collection (e.g. venipuncture performed after the last van pick-up)
- Biochemistry samples (lithium heparin and serum) should ideally be centrifuged and stored refrigerated (4-8°C)
- Biochemistry samples (fluoride oxalate) should not be centrifuged; these should be stored refrigerated (4-8°C)
- Hematology samples (EDTA and citrate) should not be centrifuged; these should be stored refrigerated (4-8°C)
- Urine samples (random 'spot' and 24-hour samples) should be stored refrigerated (4-8°C)
- If centrifugation facilities for Biochemistry samples do not exist within the facility, Biochemistry blood samples may be stored refrigerated (4-8°C), but please be aware that the integrity of these samples will be compromised, resulting in spurious results, particularly (but not limited to) plasma sodium, potassium, phosphate, LDH.
- **Do not freeze blood samples** unless specifically advised by the laboratory to do so.
- Sputum samples can be refrigerated at 4^0C.

Specimen triple layer packaging

The primary receptacle, containing the infectious substance, must be watertight, and impermeable to the substance held within (i.e. leakproof – for liquid, or sift-proof – for solids). The primary receptacle should be appropriately labelled as to content.

- A second watertight, leakproof or sift proof container should then be used to enclose and protect the primary receptacle, and its absorbent material. Absorbent material should be sufficient to soak all sample in case of spill.
- A third, outer layer of packaging is used to protect the secondary container from physical damage while in transit. It must therefore be of an appropriate strength for the weight, size and composition of the inner packages to be protected. At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm.

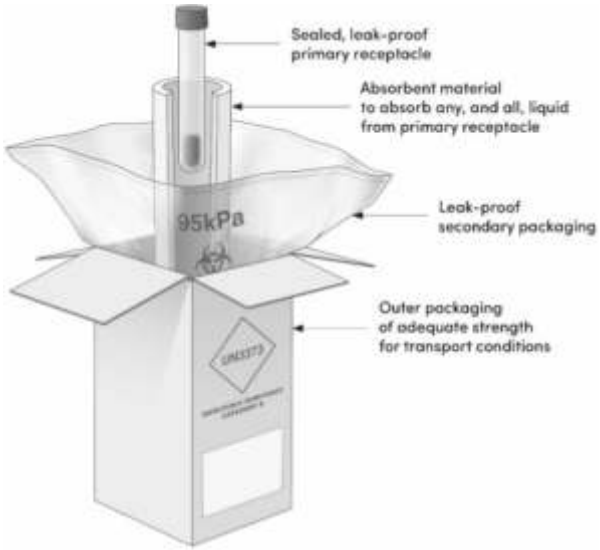


Figure 22: Triple Layer Packaging (Gli Manual)
Source: https://stoptb.org/wg/gli/assets/documents/GLI_practical_guide.pdf

Steps of Sputum packaging

1	2	3	4	5	6
					
Seal tube and cap interface with parafilm tape (multi-layer)	Wrap with tissue / absorbent paper (multi-layer)	Tight with rubber band		Put tubes in zip polybag and again tight with rubber band.	Pack sample A and B separately
7	**8**	**9**	**10**	**11**	**12**
					
Sample A and B should again be covered by another zipped polybag.	Pack Annexure –15A form separately and keep away from specimen.	Layer the thermacol box with ice gel pack (kept at -20 for two days before use)	Put sample tubes on ice gel pack.	Put another gel pack over sample tubes.	Seal the lid of box with brown tape. Biohazard Sign and proper address label.

Figure 22: Triple Layer Packaging (GLI Manual)

References:

1. Guidance on regulations for the transport of infectious substances 2021-2022. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. https://tbcindia.gov.in/WriteReadData/ l892s/8368587497Guidelines%20for%20 PMDT%20in%20India.pdf.

SAMPLE RECEIPT

Routine samples

Specimens are normally transported to lab in a rack or received courier/ postal packets. On arrival in the laboratory following steps must be taken

1. **Specimen Registration**: may be done by laboratory technician or registration clerk.
2. Remove the sample from Rack or from packet.
3. Check that the tube is properly capped and check for any leakage of specimen.
4. Read the patient's full name from the request and crock check that sample is labeled with the same name. In case of discrepancy/ unlabeled treat as rejected sample.
5. If the labelling is satisfactory record patient in register and LIMS and assign a unique number. And record unique/barcode number into sample tube.
6. From the request of ascertain what tests are required. Record request of any additional test in a special register.
7. Recheck that sample is correctly labeled with patient's name that laboratory number has been correctly assigned.
8. Rejected samples also need to be recorded in the register/ LIMS.

On call samples

- These samples are received after normal working hours and during weekends.
- The technician/ technologist must enter details in a special register and allocate a consecutive laboratory number from that register/ LIMS.
- On the next working day, a record on call tests will be entered in the register and numbered as for routine samples.

Additional tests

If the request is for a special test, check the correct sample has been provided. Place the specimen in a dedicated rack and take with request from the appropriate section of laboratory.

Urgent tests

These test requests may be from an OPD, accident and emergency cases, obstetrics, surgery or neonatal units. The processing of urgent tests has priority over other samples. On arrival they must be immediately entered in the register. Specimen tube and request form must be clearly marked (Red label or red ink) and immediately taken onto testing bench. Test must be carried out without delay.

Rejection of Specimen

There are several reasons of a specimen may be unacceptable. Only a senior technologist/ microbiologist/pathologist may reject the sample.

- **Unlabeled or incorrectly labeled sample**: Inform the test requester about the error, request for another sample.
- **Leaking Sample:** In case of minor amount of sample loss, sample may be treated as potentially hazardous. A new request form must be raised if form get contaminated. Sample may be processed. If a large volume of sample leaked out. Sample should be discarded as the result may be misleading. Request for repeat sampling.
- **Outdated samples:** if sample delayed in transportation, test requester should be informed immediately. Obtain a fresh specimen. In case fresh specimen and prompt delivery is not possible; process the specimen. Keep note in report about delayed receiving in lab.
- **Unsuitable specimen**: some tests are either performed on plain blood or anticoagulated blood. Reliability of test is affected if inappropriate samples were tested.
- If clotted specimen received must immediately inform to doctor/test requester
- Insufficient amount of blood- inform the doctor/test requester.
- Excessive anticoagulant- inform the doctor/ test requester.

References:

1. SEA/HLM/320, guidelines on standard operating procedures on Hematology, WHO


राज्य स्वास्थ्य एवं परिवार कल्याण संस्थान, उ0प्र0
SIHFWUP
उत्तर प्रदेश सरकार
ज्ञानादूते न मुक्तिः