Health Systems Transformation Platform

Competency Based Training Manual for In-Service Medical Laboratory Technologists in Primary Health Care Settings

A Participant Guide

HSTP
Health Systems
Transformation Platform

9 788194 919438

Contact

In case of any queries or suggestions, contact Mr. Sanjeev Kumar, Research Specialist, HSTP, at Sanjeev.kumar@hstp.org.in.

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Contributors

Dr. Malkit Singh, Former Lecturer, Medical Laboratory Technology, Department of Medical Microbiology, PGIMER Chandigarh, India

Mr. Sanjeev Kumar, Research Specialist, Health Systems Transformation Platform, New Delhi, India

Dr. Gaurav Chhabra, Additional Professor, Department of Pathology and Laboratory Medicines, AIIMS Bhubaneswar, Odisha, India

Dr. Kaptan Singh Sehrawat, President, Indian Confederation of Medical Laboratory Science, New Delhi, India

Dr. Mukund Sable, Additional Professor, Department of Pathology and Laboratory Medicine, AIIMS Bhubaneswar, Odisha, India

Dr. Shivasakthy Manivasakan, Director, Institute of Health Professions Education, Sri Balaji Vidyapeeth, Puducherry, India

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Abbreviations

Abbreviations	Full Form
LMICs	Low- and Middle-Income countries
HSTP	Health Systems Transformation Platform
CBT	Competency Based Training
QMS	Quality Management System
QC	Quality Control
QA	Quality Assurance
NIH	National Institute for Health
PHC	Primary Health Centre
CHC	Community Health Centre
MLTs	Medical Laboratory Technologists
LIS	Laboratory Information System
HIS	Hospital Information System
TAT	Turn Around Time
NABL	National Accreditation Board for Testing and Calibration Laboratories
SOPs	Standard Operative Procedures
EQA	External Quality Assessment
IQA	Internal Quality Assessment
ILC	Inter Laboratory Comparison
AMC	Annual Maintenance contract
RCF	Relative Centrifugal Force
RPM	Revolutions per minute
DDW	Double Distilled Water
ATCC	American Type Culture Collection
BMW	Bio-Medical Waste
UV	Ultraviolet
IR	Infra-Red
ID	Identity
BSF	Blood Sugar Fasting
FBS	Fasting Blood Sugar
CSF	Cerebrospinal Fluid
PP	Post Prandial

Background

Aligning competencies to deliver comprehensive primary care services at primary health care facilities is paramount. Competencies need to be built during the preservice education of the healthcare workforce. Human Resources for health-producing institutions must be aware of changing health needs. These institutions must maneuver health professional education to develop relevant competencies. Evidence suggests that pre-service education is inadequate to develop required competencies during pre-service education. Therefore, health systems must make significant investments to build the competencies of Primary health workers during service. However, interventions to improve the competencies of health workers have not been developed to provide quality healthcare services, especially in primary healthcare settings.

Consistent with other studies, Odisha Health System Strengthening diagnostic report, 2021 reports a significant competency gap among Primary health care providers, including Medical Laboratory Technologists.

Medical diagnostic laboratories are critical for providing adequate healthcare services and patient care. In India, primary Health Centres and community health centers serve a large portion of the population. It is vital to align the competencies of medical laboratory technologists to ensure that these professionals may deliver high-quality laboratory services.

Health Systems Transformation Platform collaborated with the Government of Odisha, All India Institute of Medical Sciences, Bhubaneswar, and Indian Confederation of Medical Laboratory Science, New Delhi, to build the MLTs' Competencies in Primary Health Care Setting. For this, HSTP and its collaborative developed the Competency assessment framework and assessed the In-service MLTs' competencies in Sample districts. Based on the assessment findings, the HSTP-led collaborative developed this "Competency-Based Training Manual for Participants" to build competencies among the in-Service MLTs in primary healthcare settings.

Contents

Introduction

Office of human resources development, NIH, USA has explained, "Competencies as the knowledge, skills, abilities, and behaviors contributing to individual and organizational performance Knowledge refers to information acquired through experience, study, or investigation. It represents the understanding that an individual has acquired over time. Skill, on the other hand, is the outcome of repeatedly applying knowledge or ability. It is developed through practice and refinement of one's abilities.

Ability refers to an individual's inherent potential to perform mental or physical actions or tasks. It is a natural aptitude that can be honed and improved upon. Behavior represents an individual's observable reactions and actions in response to a particular situation. It is how an individual behaves or conducts themselves in different circumstances. The desired level of proficiency for each competency varies depending on an individual's position and the specific needs of the organization they are working in.

Behavior represents an individual's observable reactions and actions in response to a particular situation. It is how an individual behaves or conducts themselves in different circumstances. The desired level of proficiency for each competency varies depending on an individual's position and the specific needs of the organization they are working in.

Very simply, competency is the ability of someone to carry out a given work efficiently.

What is a Clinical laboratory?

Clinical laboratories are healthcare facilities that offer a diverse range of laboratory procedures and tests. These tests are crucial for physicians to diagnose, treat, and manage patients accurately. These laboratories are staffed by medical technologists or technicians, also known as clinical laboratory scientists in some countries, who are specifically trained to conduct various tests on biological specimens collected from patients.

Preferably, these laboratories are situated within or near hospital facilities, providing easy access to physicians and their patients. The clinical laboratories can be classified as mentioned below:

According to ownership

- Government-owned, usually part of hospitals and medical
- Private facility, privately-owned medical/healthcare institution

According to function

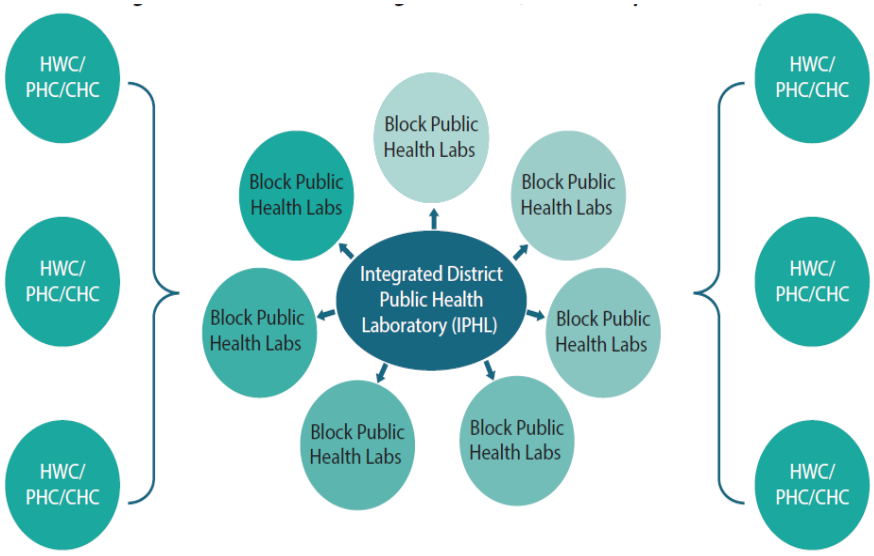
- General clinical laboratories that provide common diagnostic laboratory tests;
- Specialty/Reference laboratories that provide disease-specific diagnostic and confirmatory tests;

According to Test Specialization - Provide tests in a particular field of interest listed below:

- Clinical Chemistry
- Clinical Microbiology and Microbial Serology
- Hematology
- Blood banking and Serology (Immunohematology, Transfusion Medicine)
- Histopathology and Cytopathology
- Molecular Biology
- Public Health Laboratories perform tests such as water analysis, environmental substance testing, and other public and ecological health tests.

Laboratory Network in Public Health System:

The District Hospital's Integrated Public Health Laboratory is the central point for blocking public health laboratories. It can conduct tests that are not conducted at the block level. Likewise, the Block Public Health Laboratories (BPHL) can function as the central point for Health and Wellness Centers (HWCs) and other facilities located at lower levels.



Downward Linkages of IPHL (Hub and Spoke Model)

National Reference Laboratories, also called the central level, play a key role in managing and overseeing the laboratory network. They are responsible for implementing policies and programs, conducting training and development, monitoring and evaluating performance, and conducting research. These facilities offer routine and specialized laboratory testing services, including introducing and gradually implementing new diagnostic tests.

Clinical Laboratory Workflow

To understand the importance of competency in a Clinical Laboratory, we need to under how a clinical laboratory works:

S.N.	Step	Expected Errors during
1	Sample input to the laboratory	Collection, Labelling, preservation
2	Sample Transportation	Transportation conditions
3	Sample processing	Preparation, processing using various methods, consumables, and equipment
4	Data (Report) Generation	Reading, rough data, final data
5	Report Validation	Decision-making and authentication.
6	Report Release	How it was released, two of whom
7	Evidence (Report) based Treatment	If the report is accurate, treatment will be OK; otherwise, it may lead to mistreatment.

As, almost every step in processing the sample, if not done properly, it will definitely affect the test results on which the patient's treatment relies.

- A Medical Laboratory Technologist/Technician does all these steps.
- If a MLT is less competent or not competent theoretically as well as practically, in his/her work, the results may vary from the actual, leading to mis-diagnosis and further mistreatment of the patient putting a dent on efficiency not only on laboratory services but will also spoil the reputation of overall healthcare services provided by the concerned organization
- Low competency of MLTs will have a negative impact on health, personnel and environment safety and commerce too.

This clearly shows, that a MLT should be theoretically as well as practically competent to perform the tests so that an accurate report is generated, leading to an accurate and evidence-based treatment.

Hence, it becomes vital to assess and analyze the competency of MLTs. If something is lacking, MLTs need to be trained further so that they may work efficiently and perform accurate laboratory investigations leading to the good evidence-based laboratory diagnosis of the patient for proper treatment.

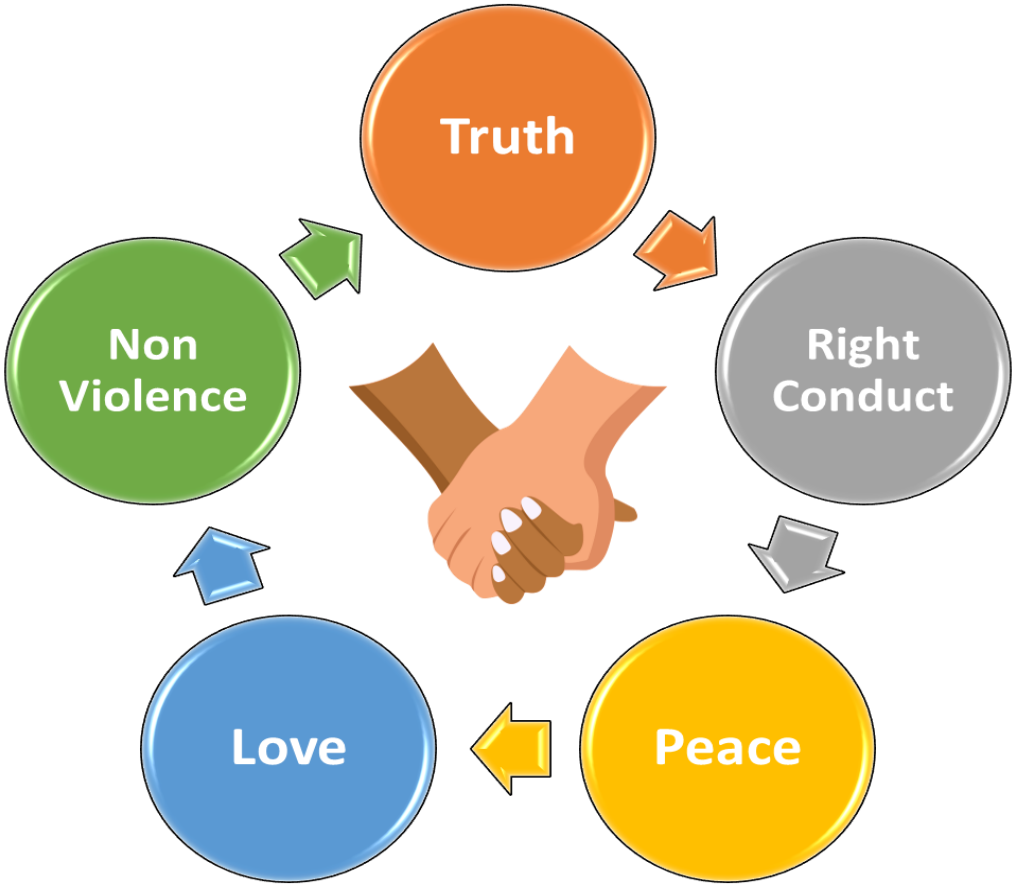
Many parameters affect the competency of MLTs to provide efficient laboratory diagnostic services to fulfill the aim of ***"the right result, at the right time, on the right specimen, from the right patient, with the result interpretation based on correct reference data and at the right price."*** They should have knowledge, a positive attitude, and an observable skill to perform. The most common are:

1. Human Values
2. Professional Ethics
3. Good Communication skills
4. Critical thinking
5. Quality Management in a Clinical Laboratory
6. Equipment: Inventory and functional quality Controls
7. Consumable: Inventory and functional quality Controls
8. Sample processing steps:
    a. Pre-analytical Phase
    b. Analytical Phase
    c. Post analytical Phase
9. Biomedical Waste Management
10. Laboratory Associated Infections: safety and infection Control

Human Values

In any healthcare setup, the visitors are either patients or their relatives, and both are already upset due to their sickness-related problems. Suppose a patient comes to a laboratory for some diagnostic tests and has been treated peacefully like a human being with good behavior & manners. In that case, his half-tension will be relieved. But if done otherwise, their pain/tension will increase.

Hence, an MLT should always understand and practice human values positively. Human values are desirable characters showing behavior with high moral values that guide us to consider humanitarian characters when interacting with others. The most important and common human values expected in all human beings:



- **Right Conduct:** It is based on human values like
  - Modesty, self-reliance, hygiene, etc. (**self-help skills**),
  - Good behavior, good manners, environment awareness, etc. (**Social skills**),
  - Courage, efficiency, initiative, punctuality, etc. (**Ethical skills**) and
  - Ownership.
- **Peace:** It contains values like equality, focus, humility, optimism, patience, self-confidence,

self-control, self-esteem, etc.

- **Truth:** It contains values like accuracy, fairness, honesty, justice, the quest for knowledge, determination, etc.
- **Peaceful co-existence:** It consists of values like
    - Kindness, compassionate consideration (Sympathetic), morality (understanding the difference between right & wrong), forgiveness, etc. **(Psychological values)**
    - Brotherhood, equality, persistence in doing something despite difficulties (perseverance), respect for others, environmental awareness, etc. **(Social values)**
- **Discipline:** It contains values like regulation, direction, order, etc.

Some routine habits to respect and implement human values in the workplace

- Unity in diversity is our slogan. We have to respect it. When a patient comes to the laboratory, we should behave with him/her humanely and respect his self-respect/dignity, irrespective of the patient's cast, religion, colour, socio-economic status, profession, etc. We should respect the beliefs of the patient.
- Respect for senior citizens should be maintained. They have given their lifetime to our society and should not be made to feel that they are now useless to society. Instead, we should learn from their experiences and make them feel honored, irrespective of their socio-economic status.
- One should have respect for his/her own senior/junior colleagues and higher authorities. We should discuss any conflicts among each other in a healthy way.
- One should be disciplined. He/she should come on in time, start and finish work on time and leave the laboratory on time unless there is some urgency.

After going through this domain, the trainees will learn as follows:

Lessons Learnt

- One should understand and implement the above-mentioned terms/factors/facts related to human values, not only in their professional life but also at every step of their routine life.
- Listen to the patient peacefully, sympathetically, and humanely.
- Be human and consider others as humans. It will fill your life with happiness, peace, and satisfaction, and you will be motivated towards human welfare to serve patients, your friends, your relatives, and your country's people.
- One should respect the diversity, dignity, values, and beliefs of patients/clients and colleagues irrespective of their cast, religion, color, socio-economic status, profession, etc.

Professional Ethics

Professional Ethics are accepted personal and business behavioral standards, values, and guiding principles established by professional organizations to guide their members in performing their duties consistently according to ethical principles. In medical laboratories, Ethics are applied in the patient's best interest. The codes of ethics for medical laboratory Technologists and Standards of Practice define professionalism in medical laboratory technology. MLTs should adhere to the guidelines with complete interest and honesty.

Some of the examples of Professional Ethics are as follows:

1. Confidentiality of Healthcare information

- Patients' data and reports are privacy sensitive, must be protected from unauthorized access, and should be confidential. All laboratory staff must promise to keep patient data confidential in words and writing.
- Patients' personal information, if leaked to private laboratories or hospitals, may be misused and exploited patients for their economic benefit e.g.
    - A report is leaked from the laboratory to others that he/she has AIDS. The patient is going to suffer the intolerable social stigma.

- A young male patient's report is leaked that he has a significantly low testosterone level and may not be sexually active. Imagine the social stigma he is going to suffer from.

- Patients' personal information, if leaked to private laboratories or hospitals, may misuse and exploit for their economic benefit.

Some Steps to maintain confidentiality:

- Test requisition forms, printouts, or files should not be unattended or kept under lock.
- Do not disclose patient medical information even to relatives without the patient's consent.
- Do not discuss confidential matters where others might overhear, like in buses, hotels, elevators, or staff rooms.
- Do not share the passwords of LIS with anyone; rather, change passwords frequently as per LIS policy.
- Avoid loud communication at work.
- Report suspicious activities to the competent authority.
- Verify patients' email addresses before emailing the results
- Make proper patient identification before giving laboratory results, either printed or via phone.

2. **Responsibilities towards patients, their attendants (Public), regulatory bodies, the profession, and himself/herself**

- When a Medical laboratory technologist receives and accepts a test requisition form along with a sample/s to perform a laboratory test, he/she, by default, enters into a contract with the patient to perform the prescribed tests with full dedication and honesty. For that MLTs should understand their responsibilities towards:
- Patients and their attendants
- Regulatory bodies govern the concerned laboratory.
- The respect of his/her profession
- Himself/herself/family

3. **Contribution toward continual professional improvement**

While working in the clinical laboratory from time to time, some of the MLT feels some problem with any of the equipment, consumables, or a process which may have an adverse effect on test results. Now there are two options for him:

- Ignore by thinking, "Chalo Jane Do, Ham ne Kya Lena."
- To think in the patient's interest and develop some idea/s to eliminate that problem to provide accurate reports to the patient.

One should opt for the second option, discuss the ideas with higher authorities and implement the change after proper validation if authorities agree to it.

4. **Compliance with applicable legislation**

The MLT should know all the legislation that governs medical laboratory technology at local, state-level, or national levels as applicable. For example, Any central national or state council requirement like:

i. Good laboratory Practice Rules,
ii. Accreditation,
iii. Notifiable diseases
iv. Pollution Control Board requirements for bio-medical waste management as per latent modifications/notification

5. **Recognizing the Competency gap and seeking action to resolve it.**

MLTs should be able to recognize his/her professional competency limitations. For example:

- You have received one auto-analyzer or semi-autoanalyzer or any other equipment, and you have not worked with that earlier; you should not even try to operate it, just thinking that you have much technical knowledge and will be able to operate it. If you are not trained to operate or are competent, talk to your higher authorities to arrange your training so that the equipment does not go out of order. Do not be overconfident. Acceptance of own competency limitations is always the best step. ***Don't try to do any work you are not authorized to do***.

6. **Practicing Informed consenting and respecting the patient's right to refuse.**

The patients always have the right to refuse any investigation or procedure to be performed on them. So, one should never do any activity without the patient's consent, i.e., one has performed the test on the patient's blood sample and released the report. Now, after the proper retaining period as per SOP, somebody asks you to give that sample to him/her for some research work as it is useless to the laboratory. Here we have to refuse state away as we cannot use that sample for anything else without the patient's consent, as it is illegal. The patient may sue us in a court of law. After the retaining period, MLTs have to discard the samples as per BMW management rules.

7. **Participate in continuing education/training programs.**

Training and retraining have always proven to maintain and improve a person's competency in his / her profession. This training is provided through refresher courses to be conducted by authorities from time to time as medical laboratory services are advancing and adding newer and improved techniques, day by date. Hence:

- Medical Laboratory Technologists should be given chances to attend CMEs and conferences to uplift their knowledge and competency.
- The MLTs should also develop their interest in attending and learning through CMEs, workshops, and conferences, maybe using their expenses. It will increase one's professional value. Sometimes, it happens that a new MLT joins the laboratory which is trained with present professional knowledge, and the senior person has older knowledge and has not attended any refresher course, CME, workshop, or conference then the older person will feel inferiority complex which sometimes becomes the reason for conflicts among them.

8. Roles, responsibilities, and limitations

He/she should be very clear about his/her roles, responsibilities, and limitations of himself/herself and his profession. One should stick to those until an additional responsibility is given. To only that to which one is entitled.

Lessons Learnt

To increase one's professional competence, one should know, understand and implement professional ethics at his/her workplace throughout his professional career. Some important learnings are:

- MLTs should keep patients' data confidential and un-accessible to unauthorized persons so that the patient does not suffer social stigma or is not exploited for monetary benefits.
- MLTs are responsible and answerable to laboratory clients, regulatory authorities, the MLT profession, and even their families. We should understand and take care of our responsibilities with happiness and honesty.
- To consider every patient, senior, and junior colleague as a human being and maintain their self-respect/dignity irrespective of one's cast, religion, colour, socio-economic status, profession, etc.
- To apply the mind and find ways to improve the laboratory professionals' competency and laboratory services to serve the ailing humanity in a better/improved way.
- Always follow the regulatory guidelines related to medical laboratory services and professionals.
- Never be overconfident; work as per one's best competency and also should be able to recognize and accept the limitations of his/her own competencies and work on competency improvement.
- Never forget to obtain the patient's informed consent before the procedure wherever required and respect a patient's right to refuse.
- All the MLTs should attend CMEs, Workshops or refresher training, etc., to uplift their professional knowledge and competency regarding the latest technologies.
- One should know and understand his/her professional roles, responsibilities, and limitations and should stick to those until asked to do by competent authorities.

Good Communication Skills

The MLT should be able to communicate effectively with patients/clients, colleagues, and other healthcare professionals in the local or regional language with respect to:

Active listening:

- Active listening refers to focusing all your attention on what someone is saying rather than simply hearing their words without much thought or consideration.
- Acquiring and honing the skill of active listening requires practice, but it may not be easy to become proficient in it. Mastering active listening takes time and patience, as it is a skill that needs to be developed over time.

Suppose the MLT does not listen actively to "what the patient is saying." In that case, he/she may miss some necessary information that might be important for testing or test report interpretation.

Verbal communication:

- Verbal communication is oral communication with words that you or others speak out loud.
- One may work well in doing his job, but if he can't communicate with others, at least in the local or regional language, it may lead to miscommunication, and further MLT's impression also goes down. The patient will not be satisfied.

So, one should be able to speak the local or regional language and understand the same language when others speak.

Non-Verbal communication:

- Nonverbal communication is "the act of conveying information without the use of words."
- This might involve using specific facial expressions or hand gestures to make a specific point,
- it could engage the use (or non-use) of eye contact, physical proximity, and other nonverbal clues to pass a message across

Sometimes, the patient or his representative is differently abled and may not speak or hear. An MLT should be competent enough to convey his message and understand the message of the differently abled person so that massage from both sides may be used effectively.

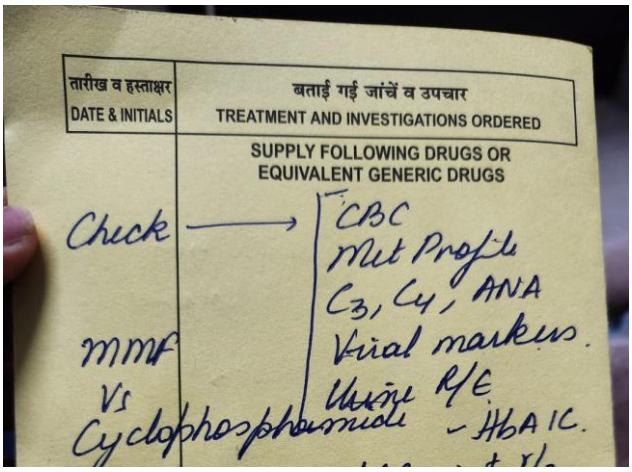
Written communication:

- It is a type of written message that two or more people exchange. Written communication is typically more formal but less efficient than oral communication.
- Examples of written communication include a pen-paper written massage, Emails, or other social media web apps.

Written communication competency is essential for an MLT because he/she may have to prepare a test report with pen and paper or register an e-mail or any other written communication method.

For written communication:

- The handwriting of MLT needs to be good and understandable
- the MLT should be familiar with the language in which the message is being written.
- Only well-acceptable and established abbreviations should be used.
- Medical Terminology: If one is not familiar with medical terminology, it affects his/her competency as either he/she may directly refuse to perform the test or there will be a delay in performing the test. Hence, one should be aware of general use medical terminologies and abbreviations. He/she should:
    - Know the terms/abbreviations used in the tests which are being done in the concerned laboratory
    - Use only those terminologies and abbreviations that are either universally accepted and used or have been approved by the hospital authorities and are well circulated.
    - Clarify any termor abbreviation which s/he does not understand in the Test requisition form or any other related document it from the concerned person and understand for future.



Good written communication skills are essential for MLT, but at the same time, the physician preparing a test requisition form should also be competent so that the test requisition written by him/her may be readable and understandable. This will help the MLT to perform his activity more effectively and in less time.

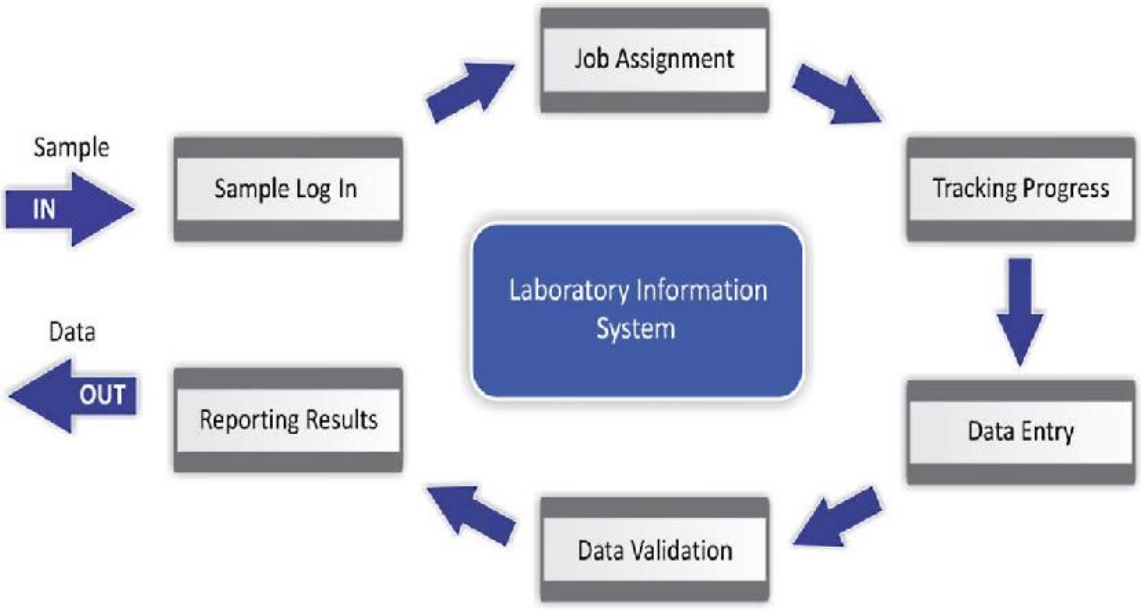
Secondly, no one can have effective interdisciplinary/intra-professional collaborative team skills if he/she is not competent in communication.

Using technology for communication

The availability of communication tools like social media platforms (such as Twitter, Instagram, and Facebook), video chat systems, and cloud-based platforms has made communication and collaboration much more convenient and efficient, both within and outside the workplace. An important advantage of these tools is their improvement in communication processes. Technology has also played a role in enhancing workplace efficiency and productivity.

In the clinical laboratory, the use of a Laboratory Information System (LIS) alone or as a part of a Hospital Information System (HIS) has been used widely and has gained popularity. This technology has significantly reduced many Pre & Post analysis errors.

Lab Information system (LIS)



The LIS system has now made communication between physicians and laboratory Personnel very easy, even without talking to each other. The sample is submitted to the laboratory, and after processing, the validated report reaches the physician's computer without delay. No manual transportation of test reports is required. Hence, to achieve turnaround time (TAT) and reduce laboratory work and data maintenance, one should be trained on usable telecommunication tools, especially lab Information Systems (LIS), wherever available.

Communication in Conflict Management:

Sometimes, out of some misunderstandings or miscommunications, conflicts arise among the laboratory staff at any level. The MLT should be competent in listening to the problem, understanding its cause, and solving the conflict for the smooth functioning of the laboratory services. Following are some requirements for conflict management that the MLTs should adopt:

- Accommodating others,
- Developing adaptive skills like daily routine activities that include talking, eating, safety, and walking.
    - A competent person, in general, does not depend only on inherited skills but develops new skills required as per their profession or daily life like "How to behave with people"?
- avoiding the conflict,
- collaborating,
- healthy competing,

- compromising
- listen to others patiently

Conflict management techniques include

- changing organizational structures to avoid built-in conflict,
- changing team members,
- creating a common "enemy," using majority rules, and
- problem-solving after an unbiased root cause analysis of the conflict

Identifying Barriers to effective communication:

Regardless of the type of communication, if professionals don't communicate effectively, it continuously decreases his/her competency. Under the circumstances, one should always identify the barriers to effective communication to improve communication skills. Some of the recognized barriers to effective communication are as follows:

- Dissatisfaction or Disinterest with One's Job
- Inability to Listen to Others
- Lack of Transparency & Trust
- Communication Styles (when they differ)
- Conflicts in the Workplace
- Cultural Differences & Language

Lessons Learnt

- **T**o communicate effectively with patients/clients, colleagues, and other health care professionals in local or regional language with respect to:
    - Active listening
    - Verbal communication
    - Non-verbal communication
    - Written communication
    - Conflict management
    - Identifying barriers to effective communication
    - Using technology appropriately to facilitate communication
- To understand medical terminology used at least related to the functioning of Medical Laboratories
- To understand and use the abbreviations commonly used in medical terminology
- To demonstrate effective interdisciplinary/interprofessional team skills through
    - Communication
    - Collaboration
    - Role clarification

- To demonstrate adaptive skills when interacting with patients
- To develop a good working environment in the work area

Critical thinking

Critical thinking is the ability to analyze information and form a judgment effectively. To think critically, professionals must be aware of the following:

- Your own biases
- Assumptions and
- The standards to be applied while evaluating the sources

Below is the ultimate Cheat sheet for critical thinking as displayed by 'The global digital Citizen Foundation,' a handy tool for critical thinking.

Work in the medical laboratory has undergone a significant change, especially regarding technology.

- High tech fully or semi-automated equipment is there to be operated by MLTs
- In addition, Laboratory Information Systems (LIS) have further changed the required qualification, experience, and competencies of laboratory Technologists/technicians.
- MLTs sometimes need to consider modern-day mechanics with very complex instruments.
- They need to think critically and make decisions accordingly.
- Troubleshooting and problem-solving are no longer left up to the laboratory managers.

- The workplace needs MLTs who can analyze a situation and make informed decisions that they need to solve the problem.
- The MLTs' exceptional communication and interpersonal skills are needed more than ever, being an essential part of the healthcare system.
- Nancy Lemelin, an MLT Educator, has raised the concern about critical thinking among MLTs and felt that being analytical personnel following competencies related to critical thinking is vital for today's MLTs
    - Logical reasoning.
    - Problem solving.
    - Objective judgment and
    - Decision making.

The following are some critical aspects that are important in a competent MLT:

Able to engage in reflective practice, which is 'learning through your own experience and developing new improvements ideas of self and professional activities:

- Teach other personnel of your cadre after conscious analysis
- Self-assess the effect of your teaching on their learning/professional activities
- Consider new ways of working to improve the quality of professional service
- Try these ideas in practice and repeat the process

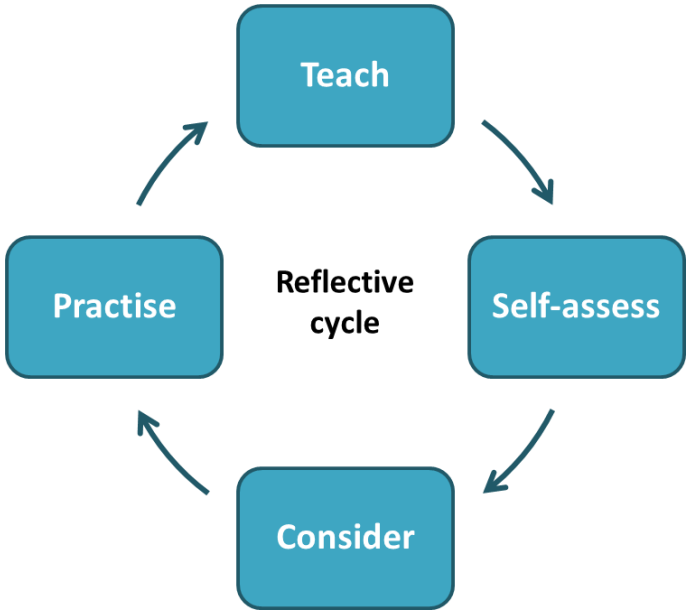


Figure: Diagrammatic representation of developing and implementing reflective practices

Able to organize work to accommodate valid priorities

The competent medical laboratory technologist/technician should be able to set the preferences while performing their duties like the priorities are to be given to:

- More sick patients

- Special requests from doctors based on Emergencies etc.
- Senior Citizens over young people
- Ladies
- Pregnant ladies over other young ladies etc

Able to ensure efficient use of 4 M (Money, Man, Material, and Minutes)

4M means effective use of available finances, manpower, all the materials of use, and time without wastage of anything. A competent MLT shall know "How to work with the least resources efficiently." If the test is not done carefully, it has to be repeated, wasting money, manpower, materials, and time also. For example,

- An elementary example is the staining procedures.
- Suppose the measured staining solution may be 0.5 ml poured onto the smear. In that case, it will give good results without wastage of staining solution.
- On the other hand, ground reality may be different. The staining solution is poured on to slide directly by tilting the bottle, wasting 2-3 ml solution.

A competent MLT always considers stopping the wastage of anything 4M

Able to demonstrate effective problem-solving/trouble-shooting strategies and initiates the appropriate follow-up e.g.

- The elementary problem is that one instrument has gone out of order. A competent medical laboratory technologist/technician should know to whom he/she should call and fix the problem as soon as possible. Even if the instrument has developed some irreparable problem, how to perform the test to which this instrument is associated, or he/she should be competent to arrange some alternative so that the test does not stop for a significant time.
- Suppose there is a problem in staining the smears. The results are not proper. A competent medical laboratory technologist/technician should be able to analyze the root cause, find the reason, and eliminate the problem. Also, he should be able to prevent the situation in the future.

Able to think about higher education and its importance in improving service quality.

- Basic education is required to perform a pre-set skill.
- If an MLT does not increase his/her professional qualification or knowledge about his/her professional skill, he/she will stagnate at one level and will not be able to move with the advanced laboratory procedures and equipment.
- A competent MLT participates in regular training and re-training, CMEs, and conferences to update his/her knowledge to improve his/her professional skill.
- Without continuously updating the knowledge through training and re-training, one even forgets the skills learned during his/her study time.

- With the advancement of technology, education levels are also increasing. The newly recruited personnel may join with updated knowledge, and seniors with less knowledge could feel inferior leading to conflict between them.

Hence, increasing and updating professional knowledge will give senior people more respect and dignity and contributes to organisational and personal efficiency.

Critical Thinking in Examination Processes

As per the government or its authorized regulatory bodies, several quality management requirements have been made compulsory. Fulfilling those requires appropriate knowledge and skills:

- To compare results with internal quality controls
- To decide about generating critical alerts.
- To identify non-conformities etc.

After going through this domain, the trainees will learn the following:

Lessons Learnt

- **T**o engage in reflective practice, stop and think about practice, consciously analyze decision-making, and draw conclusions to improve future practic**e.**
- To organize work to accommodate priorities.
- To ensure efficient use of 4 M (Money, Man, Material, and Minutes).
- To maintain decorum in the Laboratory
- To think and demonstrate the importance of service to humanity
- To think and work on patient satisfaction happily
- To demonstrate effective problem-solving/trouble-shooting strategies and initiates the appropriate follow-up.
- To think about higher education and its importance in improving service quality

Quality Management in a Clinical Laboratory

To demonstrate good competency, an MLT should be able to understand, implement and explain the concepts of a quality management system, including Quality Control, Quality Assurance, Quality improvement, consistency, reproducibility, Turn Around Time, etc., as much as he/she can, as per the prevailing facilities and requirements at the laboratory. A few important aspects are as follows:

Quality Management System

The Management system directs and controls the Quality of their services of an organization. In medical laboratories, a quality management system (QMS) plans, controls, and improves all the factors that impact the achievement of the laboratory's accurate and timely clinical test results by the laboratory and the satisfaction of the users/clients. It comprises the group of responsible persons, all the manuals, procedures, reference standards, other documents, and records that:

- Identify the patient's test requirements as per the test requisition form.
- Control laboratory operations and activities
- Record all patients and test relevant information
- Deliver the accurate and in-time test report to the patient/physician.

Quality Control

It is part of the quality management system focused on fulfilling quality requirements. Laboratory quality control aims to identify, minimize, and rectify any flaws in a laboratory's internal analytical process before patient results are released. The goal is to enhance the accuracy and reliability of the results reported by the laboratory. Quality control (QC) is a way to assess the precision of the measurement system, evaluating how consistently it produces the same result over time and under different operating conditions.

Operational activities of Quality control are aimed at monitoring the quality of laboratory services throughout the testing process and identifying unsatisfactory performance. Quality control must cover the following:

- control of errors in the performance of tests & verification of test results
- all aspects of every procedure within the department and
- it must be practicable, achievable & affordable.

Quality control sample material may be internal, i.e., a previously tested sample, or external, purchased commercially.

How to perform Quality Control (QC) in a clinical laboratory?

Quality control procedures in a clinical laboratory are performed to authenticate the quality of test results generated by it. Including QC samples in all the tests is mandatory as per accreditation agencies like National Accreditation Board for Testing and Calibration Laboratories (NABL). The control samples, also known as reference samples (Covered under reference materials) with pre-established values, are run along with the test sample under identical conditions, using the same procedure and equipment and performed by the same person in the same batch of testing. After completion of the process, the control sample results are mapped with their pre-established values. If these match, then it is considered that the test procedure has been performed accurately and the test results are accurate. For example, every commercially available diagnostic kit has a control sample in it.

The reference/control samples may be procured commercially, or the pre-tested samples with known values preserved under conditions maintaining their integrity may be used.

Quality Assurance

It is a quality management system component dedicated to ensuring confidence in meeting quality requirements. This process encompasses all measures taken to guarantee the quality of laboratory reports.

QA = IQ Control + IQ Assessment + EQ Assessment.

Laboratory Quality Assurance (QA) accomplishes the objective by implementing various activities. These activities enable laboratories to attain and sustain high levels of accuracy and proficiency, even in the face of changes in test methods and the volume of specimens tested. A reliable QA system accomplishes four key functions:

- Creates standardized operating procedures (SOPs) for every stage of the laboratory testing procedure, covering everything from receiving specimen test requests to releasing reports. This includes managing instruments and equipment and conducting performance validations.
- Specifies administrative prerequisites, such as mandatory record-keeping, data analysis, and internal audits, to ensure compliance with the established SOPs.
- Specifies TAT, corrective actions, documentation, and the personnel responsible for carrying out corrective actions, root cause analysis, and preventive actions when problems are identified; and
- Strengthening and supporting high-quality personnel performance

Quality Assessment

Quality assessment (QA) involves assessing the results obtained in the laboratory against established standards and proficiency panels. This process is used to validate quality control and quality assurance programs. Quality assessment can be carried out internally or externally through external quality assessment (EQA), which involves an outside agency examining the laboratory's performance. The widely used method of quality assessment

- **Internal Quality Assessment** (IQA): System of objectively assessing the laboratory performance on its own. In this case, the lab's staff performs the verification tests and validates their processes.
- **External Quality Assessment** (EQA): System of objectively assessing the laboratory performance by an outside agency (EQAS providing agencies). The EQA program can be organized at regional, national, or international levels. The individual results of each laboratory are confidential and typically only known by the respective laboratory and the EQA provider. However, a summary is provided that enables comparison with the overall group.

Many authorized agencies provide External Quality Assessment schemes (EQAS). The laboratory can enroll with any of them. This agency will send the control samples to the laboratory at different intervals, information about the results/test values. The laboratory will process those routinely and send the results

to the EQAS agency. Now EQAS agency will map the results with pre-established results. Based on result mapping, the agency will score the service quality of the laboratory. If the score is in the acceptable range, the test laboratory produces accurate results; otherwise, the laboratory fails.

Interlaboratory comparison (ILC)

If EQAS for the concerned test is unavailable, the laboratory can go for ILC. It will identify any other accredited laboratory (ILC Laboratory) and get one part of the split sample tested at the ILC laboratory without declaring the test results. The second part of the split sample is tested in the 1st laboratory using the same methodology. Then both results are mapped for accuracy. The split testing procedure for laboratory proficiency testing also covers the same.

Laboratory-A

(1st party - requiring ILC) -

Vs

Laboratory-B

(2nd party -accredited laboratory)

Laboratory-A will send sample to laboratory-B for testing without declaring its own results and also process the
Laboratory-B will test sample at its setup with the same procedure and send the test result to the laboratory-A for mapping and record

Accreditation agencies like NABL accept both methods (EQAS and ILC).

Accreditation

Accreditation is a process in which competency certification, credibility, or authority is presented. It is the procedure by which an authoritative body (NABL) gives formal recognition that a body (laboratory) or a person (signatory) is competent to carry out specific tasks (scope).

It is a self-assessment and an external peer assessment process used by a clinical laboratory to accurately assess their performance level to established standards and to implement ways to improve continuously.

It involves two significant steps:

- Internal audit
- External audit by accreditation agencies like NABL

Benefits of accreditation

Accreditation is laborious, but it provides the following significant benefits to the laboratory and its staff:

- National and international recognition
- Public and industry acceptance
- Assurance to clients of good laboratory practice
- Provides global equivalence
- Provides comparability in measurements
- Decision-makers can rely on test results

- Improves staff motivation
- Ensures better support in the event of the legal challenge
- Saves money by getting results right the first time.

The diagnosis and treatment of a patient are dependent on laboratory reports. Any breach of clinical sample testing and reporting quality may mislead the physician. Hence competency in maintaining and improving the quality of medical laboratory testing will enhance the role of laboratory services and laboratory professionals in diagnosing and treating patients. Medical Laboratory Professionals should know and be able to practice the principles of quality management and identify the factors that could adversely affect test results and reports in Primary Health Care Laboratories. After going through this domain, the trainees will learn the following:

Lessons Learnt

- To understand and demonstrate the concepts of quality, Quality Control, Quality Assurance, Quality improvement, consistency, reproducibility, Turn Around Time, and confidentiality in Laboratory management.
- To understand Corrective actions, Root cause analysis, and Preventive actions.
- To follow established protocols as defined in the quality policy, process, and procedure manuals.
- To use simple statistics to monitor and track the acceptability of quality control results**,** Identify documents and reports deficiencies that may affect the testing quality and the generated test report.
- To understand and perform preventive maintenance and calibration of Laboratory instruments according to established protocols.
- To participate in internal and external quality assurance activities, e.g., proficiency testing, audits, and accreditation.
- To demonstrate knowledge of inventory control by adopting FIFO (First in and First Out).
- To demonstrate information management skills, e.g., computer, laboratory information systems, and related technology wherever available
- To report promptly and accurately any hazards that he/ she is not allowed to deal with to the relevant/authorized person and warn others who may be affected.
- To Inspect equipment, structure, environment, or materials to identify the cause of errors or other problems or defects.

- To complete any health and safety records legibly and accurately.
- To report any identified breaches in health safety and security procedures to the designated person
- To identify and recommend opportunities for improving health safety and security to the designated person.
- To make suitable, accurate, and unbiased specifications of instruments or consumables for procurement as per the Laboratory's requirements.

Equipment: Inventory and Quality Controls

Many instruments/equipment are a substantial assets of a clinical laboratory. For their efficient use, training has to be given to MLTs & skill has to be developed among them for their excellent care and maintenance. Improper use/maintenance may damage the equipment, lead to loss of time & and high expenses. Even in developing countries like India, very costly and advanced equipment are being used in clinical laboratories. Before purchasing imported equipment, we need to check whether:

- Can those be repaired easily if they go out of order?
- The service engineers are available near the laboratory, so can they be repaired in less downtime?
- Are the spare parts available?

Key Point to Rember before Purchasing any Equipment
Laboratory equipment should never be purchased after due consideration as many factors are involved in their proper care and maintenance for their proper use. Before purchasing the equipment, we need to address many questions. • Is the instrument really required? • Does it suit the requirements of the laboratory? • Does it meet the safety and quality standards?

- How much space is required, and whether the space needed for this equipment is available along with other equipment?
- What is its electrical requirement?
- Can the existing laboratory personnel handle it?
- Are the supportive services available like AMC / CMC of equipment?
- Who can repair it if it goes out of order?
- How much time will it take to fix the problem?
- Are reagents, spare parts, trained persons to repair it etc. available ? (Imported Instruments)
- Advanced countries choose disposable equipment as technology is changing fast, but developing countries cannot afford that, hence choosing long-lasting & serviceable Instruments is preferred in India, if the above conditions are met.

Some Tips After Purchasing Or Receiving The Equipment In The Laboratory

- Unpacking should be done carefully (per the manufacturer's Instructions), preferably in the presence of company personnel/trained engineers.
    - The instruction manual should be read and understood. It contains operation, maintenance, troubleshooting information, and essential circuit diagrams for repair. Never lose the instruction manual; make copies for daily use and keep the original in a central file.
- It should be properly placed as per the manufacturer's instructions
- Be installed by a trained company engineer in the presence of user staff
- Calibration certifications: The company engineer should calibrate the instrument/equipment and issue calibration certificates. Preferably Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) certificates and reports.
- Company engineers should train the staff on proper operational and troubleshooting regarding functioning and general problems.

Inventory Controls of Equipment

- A file should be maintained for every piece of equipment individually at a central place, and at least the following information about the equipment should be recorded, which may include:

- Purchase & Service details like Purchase order specifications, make and model, preventive maintenance schedule etc., are required when it goes out of order, especially when condemnation in the government sector.
- Instruction Manual & Service Manual
- Calibration / Validation certificates

- Every piece of equipment should have a sticker placed on it having at least the following information:
    - Name and Unique ID
    - Serial number of instrument/equipment
    - Make, Model
    - Whether under AMC/CMC?
    - Who will repair it? Name & Contact No. in case of emergency

Operational Quality Control of Equipment

Instruments/equipment are machines that may go out of order or may not work correctly; hence their internal controls/function checks are necessary to know that the equipment is working as per requirements. We should always ensure that:

- Purchased items should consistently meet the laboratory's quality requirements.
- Calibration records should be appropriately maintained.
- Internal quality control samples should be processed with every batch of test samples, and the results be recorded and analyzed with standards.

Key Points to Care and Maintenance of Equipment

- Operating and service instructions be displayed and followed.
- The instrument should be used under the supervision of an expert person
- Two persons should be given the responsibility
- All the equipment having an impact on test results should be calibrated at the time of installation and at regular intervals after that.
    - Calibration is a process by which it is established that all parts of the instrument/equipment are showing readings similar to the reference standards instrument.
- Common maintenance calendar for all instruments should be maintained, which helps in the timely maintenance of each instrument.
- Regularly working machines *(Deep freezers, refrigerators, incubators etc.)* should be checked every morning & evening, and the temperature should be entered in the logbook.
- Switch off, Clean & Cover the instrument after every use

- The following checks are to be checked and recorded regularly:
    - Frequency of checking
    - Whether data is recorded in time
    - Comments on data / Analysis of data
    - IQC log
    - Changes made to restore accuracy & precision, if any.
- Signature with the date of the person performing these tasks
- Problems should be reported to the person in charge immediately to act immediately, and documents related to the problem & its fixation be placed in the file
- Only a trained and experienced person should repair the instrument if it goes out of order.
- Downtime recording and calculation are very important
- Downtime, if any, should – be calculated every month
- Downtime of more than 15 days per year is not acceptable
- Penalty - be imposed on the supplier as per government rules
- Any instrument under repair should be marked as '**Under repair - Not in use.'**
- If any instrument is non-serviceable, Be marked as **'Condemned / Not in use.'**
- Condemned instruments should be taken out at the earliest

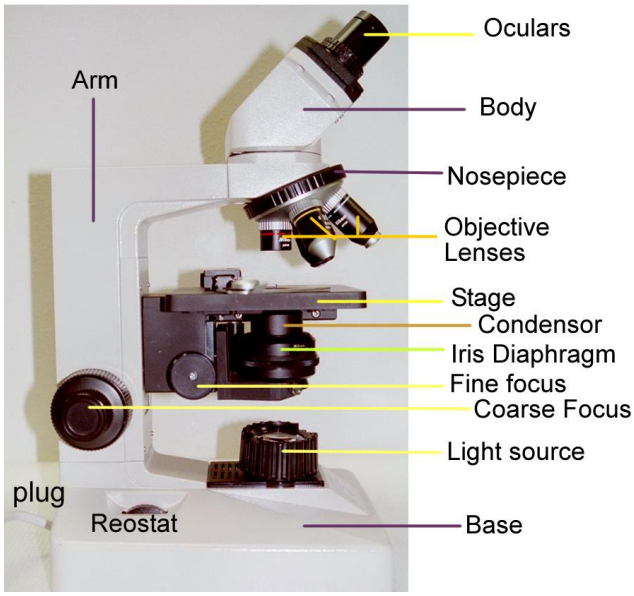
It is vital to install and operate the equipment mentioned above and keep all the records. Doing this will reduce the chances of equipment going out of order. If it goes, out of order, it can be repaired in time, and the work can be resumed.

Specific Care and Maintenance of Some Common Instruments/Equipment

There are many instruments/equipment used in a clinical laboratory, but discussing in detail each equipment is complex. Hence care and maintenance of a few commonly used equipment is discussed below:

Microscope: It is a vital instrument available in all clinical laboratories. The clinical laboratory cannot work without it. It is commonly used for hematological, microbiological, and histopathological testing.

Light Microscope Parts



- It is an expensive and delicate instrument.
- Its improper use leads to loss of image clarity.
- Fungus and dust are the worst enemies of the microscope. Hence should be kept under cover when not in use and be stored in low humidity environments, best below 60%.

Some tips for Care of the Microscope

- Carry by holding its limb with one hand, keeping the other hand under the footrest.
- Never swing it while carrying
- Keep it covered when not in use
- Blow dust particles after finishing your work & store them in a warm & dry place.Blow off dust with a rubber teat or a paintbrush.
- While working with a microscope, never pull-out slide without swinging out the oil immersion objective
- Don't use the oil immersion objective on a wet mount. If essential, then use a cover slip.
- Never touch the objective lens to the slide
- Clean the lenses before storing it
- Clean with toilet paper or old non-fluffy cloth
- Use chamois leather if available
- Never use organic solvents like ethanol, xylene etc.

Centrifuge Machine:

1. Maintenance should be done as per manufacturers' guidelines
2. If lubrication is to be done, include it in the regular maintenance protocol
3. Before using the machine, check that:
    a. Chamber is cleaned
    b. Rotor is fitted properly
    c. Soft cushions at the base of sockets are placed
    d. Cracked test tubes are not in use
    e. Solutions in all tubes are the same in quantity and weight to ensure the balance of the rotor.
4. There is a possibility of infectious spillage inside the centrifuge machine due to breakage of test tube carrying blood or other body fluid. In that case, proper cleaning & disinfection is required. Some tips for cleaning and disinfection of centrifuge machine after infectious spillage are as follows:
    a. Use a mechanical device, like forceps or tweezers, to remove the broken tube and any pieces and dispose of them in a sharp's container;
    b. Remove the tube adapter from the rotor to a container that can fit all pieces of the centrifuge;
    c. Soak all fragments in a disinfectant solution for 20 minutes;
    d. After soaking, use forceps to retrieve all parts from the disinfectant solutions to be washed and rinsed;
    e. Before cleaning the interior of the centrifuge, be sure that all visible pieces of glass have been removed;
    f. Use paper towels to soak all the liquid or blood spilled in the centrifuge's bottom. Repeat this step until all liquid has been absorbed;
    g. Dispose of all contaminated paper towels in the Biohazard Waste Container (Yellow bucket);
    h. Spray disinfectant in the interior of the centrifuge and let it stand for at least 10 minutes;
    i. Wipe the surface of the centrifuge twice with paper towels wet with distilled water and twice with 70% ethanol or isopropanol;
    j. Place material in the Biohazard Container (Yellow Bucket);

k. Soak the tube adapter in disinfectant for 20 minutes. Rinse, let it dry, and spray with 70% ethanol or isopropanol;

l. Remove gloves and dispose of them in the Biohazard Waste Container;

m. Remove lab coat if contaminated, and place in Biohazard Container or Contaminated Laundry Container;

n. Wash hands thoroughly with soap and water.

For standardization of the centrifuge machine, calculate Relative Centrifugal Force (RCF) by using a nomogram provided with equipment by the manufacturer. One may even calculate it.

Relative centrifugal force (RCF) refers to the force applied using a centrifuge. To convert revolutions per minute (RPM) to relative centrifugal force (RCF) or g force, use the following formula:

$$RCF = (RPM)^2 \times 1.118 \times 10^{-5} \times r.$$

Where:

RPM is Revolutions per minute

r *is the radius* expressed in centimeters

Weighing Balance

It is also very sensitive equipment and needs much care. It is susceptible to vibrations and leveling.

Following are a few tips for proper maintenance of a weighing balance:

1. Select a balance that suits your requirement
2. It should be placed in a firm place to minimize disturbance
3. The surface should be level
4. Use a watch glass or a piece of paper. Never put material directly on the pan.
5. Load or unload the pan only when it is arrested
6. If standard weights are to be used, pick those always with forceps
7. Always balance the empty pan to zero before use
8. Avoid spillage of any fluid or powder, especially hygroscopic ones.
9. Clean properly after use with dry non-fluffy cloth or tissue paper.

Cooling equipment

Any cooling equipment releases much heat from its compressor, so keep it in a properly ventilated room and at least one foot away from any wall or other obstructive article. Mainly, deep freezers and refrigerators are being used in Clinical laboratories. Following are some tips to take care of cooling equipment:

- **Deep freezers**
    a. Don't open it frequently
    b. Fix a time – Open once a day
    c. Maintenance of daily temperature record.
    d. The data to be maintained should at least include the Date, time (AM/PM), temperature, name, and supervisor signature.
- **Refrigerators**
    a. Regular cleaning and defrosting if an automated defrost system is not available
    b. Purchase two small refrigerators instead of one bigger to save precious chemicals; if one goes out of order, the other can be used to save precious perishable articles like standard solutions diagnostic kits etc.
    c. Always use different refrigerators for contaminated and non-contaminated articles.
    d. Like deep freezers, refrigerators also should be placed with one feet gap from the wall.

pH Meter

It is also one of the critical instruments in a clinical laboratory.

pH meter

Ill maintained electrode of pH

- It is temperature, inclination/slop sensitive. Hence,
  - if the test solution's temperature differs from the ambient one, allow the solution to come to ambient temperature before testing pH on the pH meter or use a pH meter with an in-built temperature probe to adjust it according to the temperature.
  - While cleaning /dusting, don't change its position. If changed, then recalibration required.
- Prefer pH meter with temperature sensor and auto-calibration.
- The electrode is very delicate and is fixed in a recommended plastic case.
- The electrode should always be kept dipped in DDW to be changed every day.

Hot Air Oven

It is used for drying and sterilizing glassware, not for plastic ware or anyone other heat-sensitive/labile article. Usually, regular cleaning is the main requirement for its maintenance.

However, its functional efficacy should be tested regularly. For efficacy testing, anyone of the following three methods is applied. If it is to be used for sterilization, the 3rd method (Biological indicator) should be preferred.

- Temperature chart records should be maintained and analyzed occasionally or as per SOP.
- Use a chemical indicator in a tube known as Brownie's tube No. 3. Which turns Red to Green if the required sterilization temperature is achieved, i.e., 180°C for 1.0 hours.
- Use of Biological Indicator. Geo-Bacillus subtilis var niger ATCC9372's Spores and the article load are kept inside the hot air oven. After completion of the sterilization cycle, the spores are taken out and subject to culture in the incubator at 37°C for five days. If the spores are still living, they will grow, which means the hot air oven is not working correctly. If they don't grow, it means it is working satisfactorily.

Efficacy testing of a Hot air oven:

- Comparison of daily temperature record chart with master chart prepared at the time of commissioning of equipment
- Thermocouple validation & calibration quarterly & yearly as per Health & Social security (1980)
- Daily use of Browne's tube No. 3. Turns green from red if sterilization is satisfactory.
- At least once a month, the hot air oven's efficacy should be checked using a biological indicator, i.e., Geobacillus subtilis.

In the case of a hot air oven, the biological indicator is used as in autoclaved with the same procedure except that the incubation of the ampule is done at 37°C instead of 55°C as Geobacillus subtilis grows at 37°C optimally.

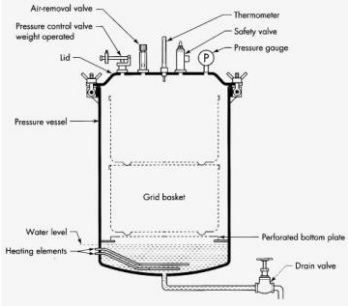
Autoclave

Autoclave is an essential piece of equipment for all levels of clinical laboratories as this is required for sterilization for microbiological work and to sterilize infectious biomedical waste like clinical samples and culture before handing it over to the BMW agency for final disposal as per prevailing BMW rules.

Autoclaves are of many types, but as per inclination, they are divided into two types, i.e., Vertical and Horizontal.

Vertical Autoclaves

Vertical autoclaves usually comprise single jacketed and positioned vertically. They are loaded from the upper side. Water to generate steam and the articles to be sterilized are in the same chamber but are separated with a perforated basket placed above the water level.



Smaller laboratories typically use this type, but the disadvantages of this are:

- The air inside the chamber is difficult to be replaced by steam as it is heavier than steam and forms an air pocket in the lower part of the chamber. Air is a bad conductor of heat; hence, the required temperature is not achieved in the lower part of the chamber and may leave the articles in this segment unsterilized.
- Since water and articles are in the same chamber, the sterilized material remains wet until it is removed, which is prone to contamination again.
- Water has to be changed every week or at the time of spillage
- Cleaning of chamber base from time to time is essential.

Horizontal Autoclaves

Normally used by larger laboratories. These are of many types like manual, fully or semiautomated, double door, multipurpose, pulsing or non-puling autoclaves, and many other types as per utility.

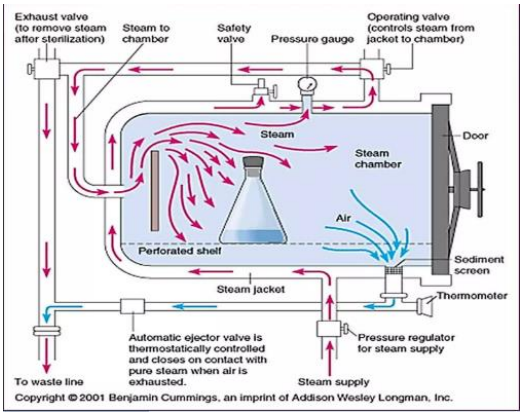


Figure: ***Diagrammatic Representation and photos of Rectangular and cylindrical Horizontal Double Jacketed Autoclave***

Double Jacketed horizontal autoclaves have the following advantages over single-jacketed vertical autoclaves:

- The air inside the chamber is replaced by steam very easily and efficiently. Hence, air pockets are not formed in the lower part of the chamber. The required temperature is achieved in all chamber parts; however, temperature mapping and other efficacy testing methods should be performed as per recommendations.
- Water is not inside the sterilization chamber.
- Only steam from a dedicated boiler outside the main chamber is supplied to the chamber.
- Steam is first supplied to the outer jacket, articles are loaded inside, and the lid is closed.
- Steam is released from the outer jacket to the inner/sterilization jacket.
- The air discharge valve is on the lower side of the chamber, which is opened to discharge air completely. The process may be manual or automated.
- After complete air discharge, the valve is closed, and more steam is pushed in till it attains the required temperature.
- After completion of the holding period, the steam is discharged from the inner chamber but not from the out jacket.
- After completing steam discharge from the inner chamber, the lid is opened and kept open for 5-10 minutes. Since the outer jacket is hot, the material dries up very soon inside the chamber only.
- When it comes out, sterilized material is dried and is not prone to immediate contamination again.

Efficacy Testing of An Autoclave:

Pressure Gauge

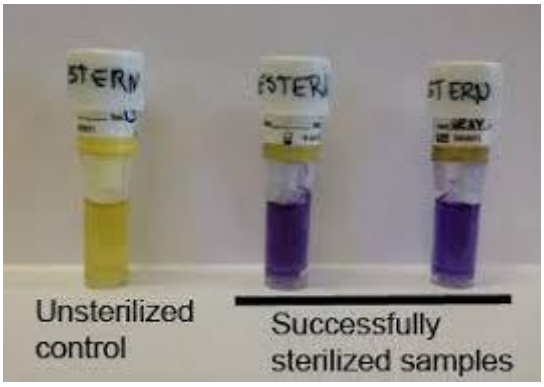
Temperature Gauge

Three indicators may be used to detect the efficacy of the autoclave process:

- Physical: pressure and temperature recording devices,

- Chemical: indicators that change colour after exposure to specific temperatures, such as temperature-sensitive tape. The colour change upon exposure to the given temperature.
  - 

- Biological: Bacillus stearothermophilus spores are used to test the biological performance of the autoclave performance, due to their heat resistance.



In a culture medium, these ampules contain a biological indicator, a bacterial (Geo-Bacillus stearothermophylus) spore suspension.

- Usually, two ampules are taken, one as a test placed inside the load and autoclaved.
- One is kept as the negative control, which is kept outside without autoclaving.
- After autoclaving, both the ampules are incubated in a dry bath at 55°C, which is the optimum temperature for the growth of test bacteria for 5-7 days.
- An unautoclaved negative control will change its colour as the bacteria in it is living and has produced acid in culture media, changing the pH indicator colour of the added to the medium.
- If the test ampule does not change its colour, it means organisms have been killed, and the autoclave is working efficiently.
- Suppose the test ampule also changes colour like negative control. In that case, it means organisms are still living, and the autoclave is not working efficiently.

While purchasing an autoclave, one should be very careful that

- the inner chamber should be made up of stainless steel, preferably grade SS 316.
- As manually operatable autoclaves are unsafe, semi-automatic or preferably fully automatic autoclaves should be purchased.

- Use of soft water for steam generation

Some Essential Tips for Good Maintenance of an Autoclave

- While using autoclaves
  - Use of soft water for steam generation
  - During sterilizing cultures or other contaminated materials, avoid the spillage of agar inside the chamber.
  - SS-made baskets (specially designed) or autoclavable plastic bags may be used to avoid spillage.
  - For liquid culture media sterilization, use heat-resistant high-quality glass bottles as containers for media.
  - Regular efficacy testing using indicator tape or if possible, biological indicators

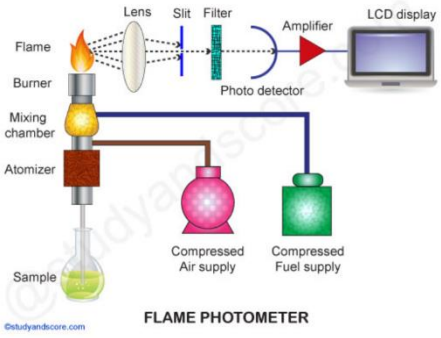
Flame-Photometer

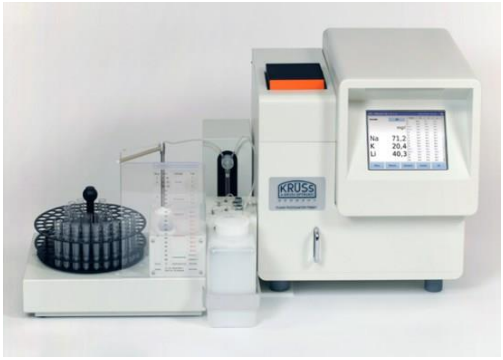
The flame photometer operates on the principle of measuring the intensity of light emitted when a metal is introduced into a flame. The wavelength of the color emitted provides information about the element, while the intensity of the flame color indicates the amount of the element present in the sample. These wavelengths are typically within the visible region of the electromagnetic spectrum. Each alkali and alkaline earth metal has its own specific wavelength.

Sr No	Element	**Emitted** Wavelength	Flame colour
1.	Sodium	589 nm	Yellow
2.	Potassium	766 nm	Violet
3.	Barium	554 nm	Lime green

4.	Calcium	622 nm	Orange
5.	Lithium	670 nm	Red

For specific concentration ranges, the intensity of the emission is directly proportional to the number of atoms returning to the ground state, and the light emitted is, in turn, proportional to the concentration of the sample.





Interferences and Limitations

The air pressure, the oxygen concentration, and the presence of oxides like Carbon dioxide, Nitrogen dioxide, and Sulphur dioxide in the surrounding air interfere with the results of the flame photometer.

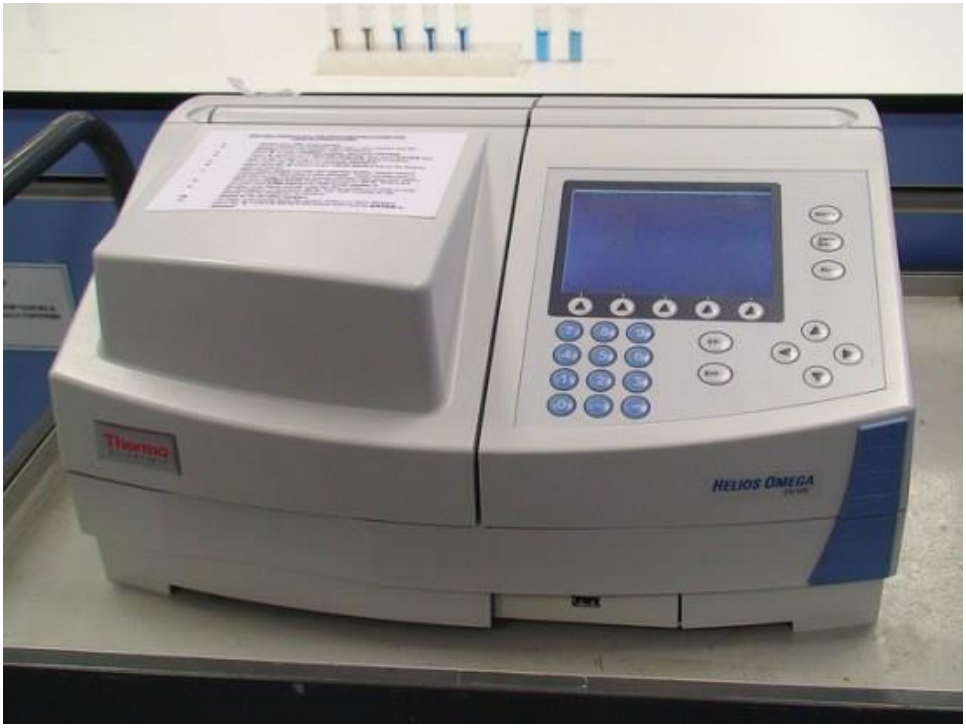
Control of Adverse Effects on Results

The above factors can be controlled via the calibration curve and standards, as the results would then be calibrated for your environment. This is why it is vital not to let a single calibration curve remain on an instrument over a few days or multiple analyses. Good Flame Photometers utilize sealed chimneys, ensuring only filtered air is passed to the flame. With the built-in compressor and gas regulation system, the finite control of a flame is vastly more achievable. Whatever type of instrument you are using, it is still essential to calibrate your instrumentation regularly throughout the day, even if you are testing the same sample type.

Spectrophotometer

A spectrophotometer is a device used for analysis that measures a sample's concentration by detecting light absorption. It utilizes a material's reflective or transmissive properties based on its wavelength. This instrument can operate in various light ranges, such as visible light, near ultraviolet, and infrared. A sample

is placed in a cuvette within the instrument to conduct the analysis. A beam of light passes through the sample, diffracts into a range of wavelengths, and the instrument measures the intensities using a charge-coupled device. After passing through the detector, The analysis results are displayed on a screen.



Chemiluminescence

Working Principles: Enzyme Immunoassay (EIA) uses enzyme-labeled antibodies and antigens to detect specific small biological molecules. This technique is based on the concept of antigen-antibody binding in immunology. The fluid sample being tested may contain various antigen molecules such as peptides, hormones, and proteins. Chemiluminescent immunoassay employs chemiluminiscence generating molecules (such as luminol derivatives) as labels in presence of H_2O_2 or OH^-, generating large quantum of light instead of producing a specific color. "luminescence" refers to the emission of light when a substance returns from an excited state to a ground state.

Benefits of Chemiluminescence Immunoassay

Chemiluminescence has several advantages over other immunoassays that use techniques such as fluorescence detection or light absorption. One of the main benefits is the high sensitivity of this technique, which enables it to detect even small amounts of the measured biological molecule. Additionally, chemiluminescence has a broader range of detection and a linear relationship between the luminous intensity and the concentration of the tested substance. An enhancer can further enhance, which helps protect the enzymes and prolongs the reaction time without reducing the light output. Furthermore, the

enhanced reaction produces intense light emission for an extended period, and the substrate needs to be added only minutes before detection.

Chemiluminescent immunoassay is developing very rapidly. It is much more sensitive and accurate than the enzyme immunoassay and fluorescence method. Chemiluminescence immunoassay has become a technique that integrates the knowledge of many disciplines. Chemiluminescence immunoassay is widely used in clinical testing, drug analysis, environmental monitoring, and other fields.

As equipment/instruments consumables are the essential components of a Medical Laboratory, understanding the functionality of equipment/instruments would be vital which trainees will learn as follows after going through this domain:

Lessons Learnt

- To prepare the equipment specifications considering the capacity requirement, space availability, electrical load, and availability of trained MLT to operate the instrument.
- To understand and maintain all the purchase, installation, operation, maintenance, and functional quality check records.
- To demonstrate the work principles of all the laboratory instruments/equipment
- To apply principles of particle analysis, commonly used in analyzer/semi-auto analyzer for blood count and others if available at respective PHC.
- To understand work principles of light measuring equipment.
- To perform risk assessment while using any equipment
- To have the concepts of calibration of equipment and its importance in laboratory services
- To maintain equipment working and its integrity through regular maintenance
- To inform competent authorities if the equipment has gone out of order
- To demonstrate functional quality check of all the available equipment/instruments
- To label the equipment/instrument with their respective unique I.D. Codes, date of purchase, date of installation, date of putting into service, date of the last calibration, and name and contact of address mechanic whom to inform in case of emergency
- To prepare and demonstrate SOPs for the use of all equipment/instruments, test procedures, and their display on workbenches
- To demonstrate all the above to his juniors for their learning and to the auditors/competent authorities as per requirement.

Consumables: Inventory and Quality Controls

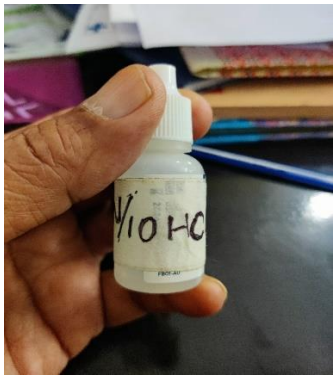
Every clinical Laboratory has many consumables: chemical, readymade, or in-house prepared reagents, diagnostic kits, etc. Competent MLTs should be able to prepare laboratory reagents, calibrators, and quality standards for quality control of various tests. These days most of the tests are being carried out by Semi/fully automatic analyzers, which use readymade kits of reagents. But still, there are some reagents or materials and chemicals which are required to be used in routine laboratory work, or sometimes these are required as a backup through manual testing when some autoanalyzer goes out of order. Hence, all the MLTs should have the following knowledge about any consumables required/used in the laboratory:

- Whether prepared in-house or commercially procured
- The specifications
- Nature
- Acceptance and rejection criteria
- Inventory control, including preservation while not in use
- How to use
- How to keep it on the workbench
- How to preserve after use
- How to discard/dispose of after expiry date or after use.

Let us discuss this one by one:

1. **Whether prepared in-house or commercially procured ?:**

The date of manufacturing and expiry of chemicals are critical issues in purchasing, storing, and using chemicals, reagents, diagnostic kits, etc. The consumables should never be used after the expiry date. The reagents are often prepared in-house, which may be less stable and not work as commercially available consumables.



The above photograph shows that 1/10 HCL is an in-house prepared chemical. Every vial of in-house prepared chemicals should contain the details of :

- Name and strength or potency of Chemical/reagent
- Date of preparation
- Expiry date, which is always validated by performing stability testing processes
- Who prepared it?

Bulk purchase of chemicals: Sometimes, the central purchase/procurement department purchases the consumable in bulk and provides it in a liquid form, as shown in the -given below:

The Carbol fuchsin solution has been supplied to the laboratory in an empty single-use plastic water bottle. This has become the stock solution of Carbol fuchsin for that laboratory. Nothing has been mentioned on this bottle except Carbol fuchsin. MLT of this laboratory fills this solution into a smaller bottle and says the filling date as the date of manufacturing and three months later as the expiry date on the label. When this

small bottle is finished or expires after three months, it will be filled from the stock bottle, again mentioning the filing date as the manufacturing date. This creates confusion about a) when the stock solution was manufactured and b) whether the solution is now working or not?

This type of act leads to diminishing the functional accuracy of the laboratory. Hence, laboratory reagents should always be procured as per the requirement of individual laboratories so that the solution's integrity is known while using as per the date of manufacturing and date of expiry mentioned on the bottle by the manufacturer.

2. **The specifications**

While raising the demand/indent of any consumable, the MLTs should consider the following:

- Whether the consumable is required?
- If required, how much quantity is needed?
- Is the specified consumable compatible with our testing system, i.e., manual or automated?
- Raising the indent should be on time so the service goes smoothly.

3. ***Nature:*** If the reagent prepared in-house, it has to be validated for its intended use. If it is commercially available, it should have the proper manufacturing date and expiry date, which has to be followed.

Acceptance and rejection criteria: There should be acceptance/rejection criteria for consumables. The consumable shall not be accepted if

- The packing is damaged
- the label is damaged
- no expiry date is mentioned except for some chemicals like NaCl
- not transported as per required climatic conditions, i.e., a consumable is perishable and was to transport at 2-8°, and when received in the laboratory, it was at ambient temperature. Hence temperature inside the packing needs to be checked before accepting the material
- Are the consumables as per the supply/purchase order?

The consumable shall be accepted only if

- It meets all the requirements as per specifications/requirements.

4. **Inventory control, including preservation while not in use**

After accepting the consumables, those should be kept under the required/recommended conditions. If these are to be kept at 2-8°C, they should be shifted to the refrigerator immediately to maintain the cold chain and functional integrity of consumables. The refrigerator or deep freezer should be on a backup

electrical line so that it should not stop working. The inventory refrigerator should be different from the refrigerator consisting of contaminated materials.

All consumables should be entered into inventory registers or the LIS system if it works and is validated. The entries should include:

- Name of article
- Date of receipt
- Purchased/procured from
- Supply order date and number
- Supplier's address
- Previous balance
- Total balance, including present supply.
- Issued to whom and how much?
- Balance after every issue.

5. How to use?

A competent MLTs shall know 'How to use' for all the consumables in the diagnostic kit. Suppose one diagnostic kit has five reagents that are in use and unexpectedly, one of the five is finished. In this case, it is advisable to replace the whole diagnostic kit with new one rather than replacing one reagent from another kit.

6. **How to keep it on the workbench, and how to preserve it after use?**

The workbench should be clean and should not have anything other than required for the concerned test. All the consumables needed for the test to be conducted should be placed on the workbench serial-wise. The perishable consumables should be removed from the refrigerator just before their use and kept back in the refrigerator immediately. They will lose their functional integrity if kept on the bench under ambient conditions for longer periods.

7. How to discard/dispose of the consumables after the expiry date or after use?

All the consumables/vials containing consumables should be discarded after use or expiry as per existing Bio-Medical Waste management rules. Some consumables require neutralization before discarding (e.g. Sodium hypochlorite, Glutaraldehyde) into the drain, etc.

The consumables are an integral part of any laboratory. Good quality reagents and storage are vital for good laboratory test results. Hence, after going through this domain, the trainees will learn the following:

Lessons Learnt

- To deal with in-house and commercially available reagents separately.
- To prepare reagents, calibrators, standards, and quality control materials
- Acceptance and rejection criteria on receipt of consumables at the laboratory
- What and how to document and record regarding consumables?
- To demonstrate the shelf life of ready-to-use commercially procured and in-house materials prepared materials and reagents.
- To follow the principles of FIFO
- To check the functional quality of all reagents / diagnostic kits/cards/ chemicals used in the Laboratory.
- to arrange and store positive and negative controls and their importance in all tests.
- Importance of Expiry dates.

Sample Processing / Clinical Test Phases

From receipt of clinical sample to release of test report, the process is divided into three phases; 1. pre-analytical; 2. Analytical, and 3. Post-analytical phase.

Pre-Analytical Phase

The pre-analytical phase includes various steps, which will be discussed below. The MLTs should be competent in the following:

Understanding the relevant information provided on test requests on requisition forms is essential for interpreting results, critical alerts, or recording purposes.

- The requisition should come in a separate format, not on the physician's Medicine prescription card.
- A competent MLT shall check whether all the following information is available on the requisition form.
    1. Name of Patient (Clearly mentioned)
    2. Patient ID (Sr Number or Registration number etc.)
    3. Age & Sex
    4. Presumptive/confirmed clinical diagnosis
    5. Whether the patient is on some medication? If yes, what medicine?
    6. Nature of sample
    7. Collection procedure (In some cases like suprapubic collection or from urinary catheter of a urine sample)

i. Date and time of collection
ii. Date and time of sample receipt in laboratory (To be mentioned by MLT receiving the sample)

2. **A competent MLT should be able to provide all relevant information about precautions on specimen collection to the patients/clients wherever required, transportation, and storage of samples till they are subjected to processing.**

Quality laboratory results begin with accurate sample collection, preservation, and transportation to the laboratory. The sample collection should be done to ensure accuracy and safety, minimize patient discomfort, and avoid recollection of the sample.

The test results greatly depend upon the sampling conditions. Suppose a sample is collected to determine fasting blood sugar level when the patient comes after taking his/her meals. In that case, the blood sugar level will be higher than his fasting level. It will mislead the physician and will lead to a wrong treatment. Hence a competent MLT shall know various pre-sampling conditions for different tests and will guide/enquire the patient prior to sampling. He will only take the sample if the patient follows the required instructions/precautions.

Many Physiological factors affect laboratory test results. The patient himself/herself is the most crucial factor in determining the accuracy and reliability of their laboratory test results. The samples like blood, urine, stool, sputum or throat swab etc., for testing are taken from the patient's body. Therefore, it becomes essential that the patient should follow pre-sampling instructions if there are any, to prepare for the test to be done.

- Before sampling, a competent MLT shall explain and make the patient understand all the pre-sampling instructions to patients, which will affect his/her test results.
- Even before taking the samples, MLT should confirm that the patient has followed all instructions.
- The patient should also honestly inform the MLT or phlebotomist if he/she has deviated from instructions and MLT should run through the set of queries to elicit this information from the patient.
- The patient should inform the treating physician when he/she prescribes the tests or at least at the laboratory of any medications (including vitamins and supplements) that S/he is currently taking.

The MLT/Phlebotomist should inform the patient about important instructions relevant to the factors that could affect test results

For some common queries and important patient instructions, **please refer to the following:**

For most of the blood tests, the sample is collected after 10-12 hours of fasting. Alcohol consumption, smoking, tea, and coffee should not be taken during fasting. A reasonable amount of water intake is permitted.

Blood samples from diabetic patients to estimate blood sugar levels

For fasting Blood Glucose (BSF/FBS), overnight Fasting is mandatory, as mentioned above, and for post-prandial (PP) Blood Glucose level, the blood sample is collected 1.5 – 2 hrs after a normal meal, normally breakfast.

The known diabetic patients should carry tiffin box for breakfast. They should take their breakfast and routine medicines after collecting the 1st sample (Fasting). The PP sample should be given 1.5 to 2 hours after breakfast.

Urine sample for routine and microscopy:

The first-morning urine sample is usually the best for most tests, including pregnancy. Discard some initial and last portions of urine and collect the middle portion (mid-stream sample) in a container. Round, wide-mouth bottles of 25-50 ml capacity with screw caps are suitable. Fresh disposable plastic containers are the best.

Urine Microbiological examination (Bacterial or fungal culture):

The container must be sterilized and is normally provided by the laboratory. For urine culture freshly voided, the sample is the best.

24-Hour Urine Sample Collection :

This sample is usually required for excretory functions of kidneys, especially 24 hours urine protein quantification. MLTs should instruct the patient to arrange a clean and dry container with 3-4 liters capacity. At 6 AM or any other convenient hour when the patient gets up in the morning, he/she should empty the bladder and discard this urine. Collect all urine passed after that in the container. Continue collecting urine until the patient gets up the next morning and passes urine. Finally, the last urine is passed at the time when last morning 1st sample was discarded (Say 6 AM), and thereafter the urine was collected. Include this next day's sample to the total urine volume till 6 AM. Keep it in the refrigerator until it's delivered to the laboratory for further analysis. Do not add any preservatives of your own. This may interfere with certain test results.

Collection of Sputum sample

The sputum sample is essential for diagnosing some respiratory infections and malignancies. Sputum is thicker and mucoid and different from saliva in liquid form. This material is coughed up from the throat and/or lungs and is normally collected in a 60-100 ml, wide-mouthed sterile screw-capped container. An early morning sample after rinsing mouth and teeth with water is preferable as it is obtained from the respiratory passage. Normally three sputum samples on consecutive days are taken, particularly for the diagnosis of tuberculosis. Specimens should be collected preferably after a deep explosive cough.

Why is stool examination done, and how to collect stool for routine examination?

It is needed to diagnose worm or parasite infestation or for the presence of blood or certain chemicals. Ideally, The patient must not receive iron or any other metallic preparations for five days before stool sample collection. Anthelminthic drugs or barium studies should also be avoided for two weeks before the test. Any type of wide-mouth and tight screw-capped disposable container can be used.

How to collect semen for analysis?

The semen should be collected after three days and no longer than five days of abstinence. The person should pass urine and empty bladder before ejaculation. Semen should be collected by masturbation in a screw-capped plastic container and delivered to the laboratory within one hour of collection while being kept close to the body to maintain temperature during transit interval. Please also note the time of collection of the sample.

MLT should be able to confirm the patient's identity, counsel the patient, and performs venipuncture and capillary blood collection to obtain appropriate samples for laboratory analysis.

The MLT should always confirm the patient's identity so that the sample is taken from the right patient. After confirming the identity, the patient should be asked to sit relaxed on the chair. He/she should be informed about how the blood sample will be taken and how much pain may be there so that the patient may be prepared and not react adversely when the needle is inserted in his/her vein to avoid needle stick injury.

MLT should be able to demonstrate the types and use of procedures or tools for sample collection, like vacutainers for blood samples:

The competent MLT should know all the tools required for blood and other sample collection, including the specifications of tools like size and thickness of needle or lancet. The sample collection containers or tubes Etc

Able to demonstrate what type of sample is to be collected for a required test like serum plasma or whole blood etc., and how to collect:

- a throat swab from a diphtheria patient
- a sputum sample from a case of respiratory infection, particularly a suspected case of tuberculosis
- A blood sample from a suspected case of diabetes, like Fasting blood sugar, and a Postprandial sample

Able to demonstrate skin disinfection procedures before taking any blood sample or otherwise wherever required

A competent MLT shall know and demonstrate different types of skin disinfectants like alcohols, alcohol-based skin disinfectants, and iodine-based skin disinfectants i.e.

a. Ethanol
b. Isopropyl alcohol
c. Chlorhexidine
d. Alcohol + Chlorhexidine
e. Povidone-Iodine

It is also important to know and demonstrate:

a. The way to apply different skin disinfectants
b. Their effective concentrations
c. Contact period and
d. Precautions to be taken while using each of them

Some factors influence the outcome of laboratory results during sample collection and transportation. These include:

- knowledge of staff involved in blood collection;
- use of the correct gauge of the hypodermic needle to prevent hemolysis or abnormal results;
- the anatomical insertion site for vein puncture;
- the use of recommended laboratory collection tubes;
- patient–sample matching (i.e., labeling);
- transportation conditions;
- interpretation of results for clinical management.

The sample should be collected from the right patient and under the right test requisition form below given Do's and Don'ts keeping in view the Infection prevention and control practices:

Do's	Don't
DO carry out hand hygiene (use soap and water or alcohol rub), and wash carefully, including wrists and spaces between the fingers, for at least 30 seconds (follow WHO's	DO NOT forget to clean your hands
DO use one pair of non-sterile gloves per procedure or patient	DO NOT use the same pair of gloves for more than one patient DO NOT wash gloves for reuse
DO use a single-use device for blood sampling and drawing	DO NOT use a syringe, needle or lancet for more than one patient
DO disinfect the skin at the venepuncture site	DO NOT touch the puncture site after
DO discard the used device (a needle and syringe is a single unit) immediately into a robust sharps container	DO NOT leave an unprotected needle lying outside the sharps container
Where recapping of a needle is unavoidable, DO use the one-hand scoop technique (see Annex G)	DO NOT recap a needle using both hands
DO seal the sharps container with a tamper-proof lid	DO NOT overfill or decant a sharps
DO place laboratory sample tubes in a sturdy rack before injecting into the rubber stopper	DO NOT inject into a laboratory tube while holding it with the other hand
DO immediately report any incident or accident linked to a needle or sharp injury, and seek assistance; start PEP as soon as possible, following protocols	DO NOT delay PEP after exposure to potentially contaminated material; beyond 72 hours, PEP is NOT effective

3. Order of Draw in Blood Sample Collection:

As per WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy, Blood specimen collection is one of the most underestimated procedures in health care. Knowledge of vein selection, the order of draw, test-specific handling, storage and transportation requirements, anatomy of the antecubital area, safety precautions, alternative sites, and other factors make phlebotomy a highly technical procedure.

Irrespective of the method adopted for sample collection, while pouring the sample in vials, the order of draw as given below should be kept in consideration:

Recommended Order of Draw for Plastic Vacuum Tubes

Order of use[a]	Type of tube/usual colour[b]	Additive[c]	Mode of action	Uses
1	Blood culture bottle (yellow-black striped tubes)	Broth mixture	Preserves viability of microorganisms	Microbiology – aerobes, anaerobes, fungi
2	Non-additive tube			
3	Coagulation tube[d] (light blue top)	Sodium citrate	Binds (chelates) calcium ions	Coagulation tests (protime and prothrombin time) requires full draw
4	Clot activator (red top)	With or without Clot activator	Blood clots, and the serum is separated by centrifugation	Chemistries, immunology and serology, blood bank (cross-match)
5	Serum separator tube (red-grey tiger top or gold)	None	Contains a gel at the bottom to separate blood from serum on centrifugation	Chemistries, immunology and serology
6	Sodium heparin (dark green top)	Sodium heparin or lithium heparin	Inactivates thrombin	For lithium level use sodium heparin, for ammonia level use either
7	PST (light green top)	Lithium heparin anticoagulant and a gel separator	Anticoagulants with lithium, separates plasma with PST gel at bottom of tube	Chemistries
8	EDTA (purple top)	EDTA	Binds (chelates) calcium ions	Haematology, Blood Bank (cross-match) requires full draw
9	Blood tube (pale yellow top)	Acid-citrate-dextrose (ACD, ACDA or ACDB)	"binds (Chelates) calcium ions" and promotes red cells & platelet life(metabolism)"	HLA tissue typing, paternity testing, DNA studies
10	Oxalate/fluoride (light grey top)	Sodium fluoride and potassium oxalate	Antiglycolytic agent preserves glucose up to five days	Glucoses, requires full draw (may cause haemolysis if short draw)

ACD, acid-citrate-dextrose; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; HLA, human leucocyte antigen; PST, plasma separating tube.

[a] "1" indicates draw first, and "10" draw last (if used).

[b] Verify with local laboratory in case local colour codes differ.

[c] Gently invert tubes with additives to mix thoroughly; erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

[d] If a routine coagulation assay is the only test ordered, then a single light blue top tube may be drawn. If there is a concern about contamination by tissue fluids or thromboplastins, then a non-additive tube can be drawn before the additive tube. The PST tube contains lithium heparin anticoagulant and a gel separator; if used, draw in the order shown.

Source: Table adapted with permission from WebPath, Mercer University, United States (http://library.med.utah.edu/WebPath/webpath.html). Order is based on United States National Committee for Clinical Laboratory Standards consensus.

4. Blood sample collection:

Whatever the way the sample is collected, it should be collected in an accurate sample collection tube. When plasma or whole blood is required for the test. It must be collected in a vial containing a suitable anti-coagulant.

Anti-coagulants:

- Heparin:
  - Very good anticoagulant which inhibits the conversion of prothrombin to thrombin
  - Quantity required: 0.2 mg/ ml of blood
- EDTA (Ethylenediamine tetra acetic acid) available as K2 (spray dried form) or K3 EDTA (liquid). K2 EDTA is preferred as it is available in spray-dried form, thus preventing sample dilution.
  - Chelates calcium ions and prevent coagulation
  - Quantity required: 2mg/ ml of blood
  - Well suited to CBC and DNA based Assays
- Sodium fluoride and Potassium oxalate
  - Precipitates Calcium ions
  - Stabilize glucose in plasma, hence commonly used for Blood sugar analysis
  - Quantity required: Sodium fluoride 2.5mg per mL + potassium oxalate 3mg per ml of blood
- Sodium citrate
  - It converts calcium into a non-ionized form
  - Not satisfactory for Calcium estimation in the blood sample
  - Quantity required: 3 mg/ ml of blood or sodium citrate (3.2%) to blood ratio of 1:9

Blood Sample collection Vacuum/non-vacuum tubes (Colour coded):

There are different sample collection tubes which have universal color-coded caps for various applications. One is vacuum tubes which suck the required blood quantity automatically when fitted into a sample collection tube holder. Other are non-vacuum tubes in which blood is added after collection with a syringe. In both cases, color coding is the same and universal, which is described below:

Red Cap tubes: These are commonly known as plain tubes and do not have any additives. These tubes are used when tests are to be performed on serum.

S.N.	Cap colour	Image	Description
1	**Red**		These are commonly known as plain tubes and do not have any additive. These tubes are used when tests are to be performed on serum
2	**Lavender**		Contain EDTA and are commonly used for Complete blood cell counts. These tubes can be used to obtain: a. Lymphocytes for DNA extraction b. Plasma for nutritional analysis c. Red blood cells for other assays
3	**Green**		Contain lithium or sodium heparin and arecommonly used for molecular studies, Osmotic fragility test
4	**Blue**		Contains Sodium citrate and commonly used for coagulation studies
5	**Yellow**		They contain Silica to activate clotting of the specimen, and a gel that, forms a barrier between the clot and serum after centrifugation
6	**Grey**		Vacutainer Fluoride EDTA Tubes and Sodium Fluoride / Potassium Oxalate Tubes are used for glucose determinations.

Tube Management*:* All tubes should be arranged in a rack to avoid confusion before sample collection.

Blood Vs. Serum Vs. Plasma

- Blood is the red, liquid component of the circulatory system. It carries oxygen and nutrients to the body's cells and removes waste products. It is made up of red and white blood cells, platelets, and plasma.
- The yellow liquid component of blood is known as plasma, making up about 55% of total blood volume. The fluid part of blood carries dissolved substances such as hormones, enzymes, and waste products.
- Serum is the liquid part of blood left after the blood has clotted. It is similar to plasma but lacks clotting factors (fibrinogen and other clotting factors) and blood cells (red blood cells, white blood cells, and platelets).

Blood Composition

Whole blood comprises **plasma** (fluid portion of blood) and **formed elements** (blood cells and platelets). Plasma is the fluid portion of blood that contains proteins, ions, nutrients, hormones, antibodies, metabolites, enzymes, clotting factors, etc. Plasma is straw-colored and makes up approximately 55% of whole blood volume.

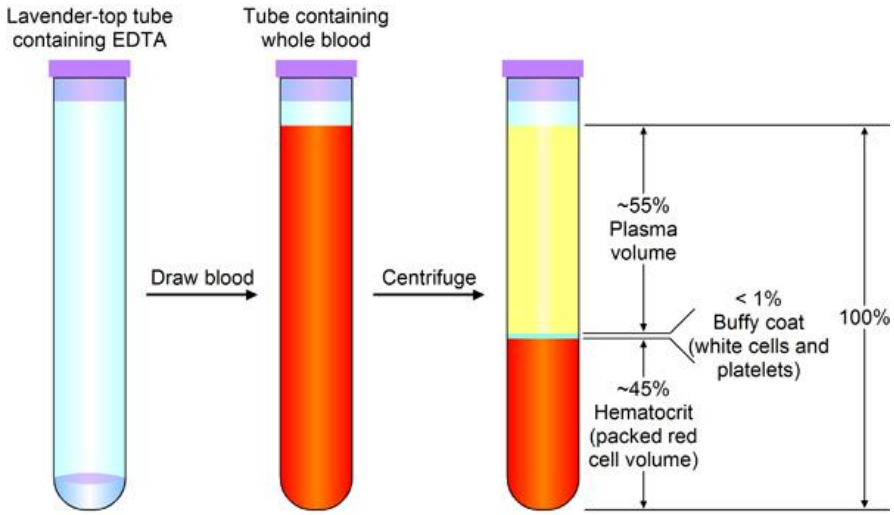
The components that makeup blood are the red blood cells (also called erythrocytes), white blood cells (known as leukocytes), and platelets (referred to as thrombocytes). When combined, these blood cells and platelets account for about 45% of the total volume of blood.

Hematocrit is the percentage of total blood volume occupied by red blood cells and is also called **packed red cell volume (PCV)**. To determine hematocrit, whole blood is centrifuged for a few minutes, separating the formed elements from plasma.

After centrifugation, three distinct regions can be observed:

- Plasma, which is ~55% of total blood volume and is the top region,
- Red blood cells (hematocrit or PCV), which make up ~45% of total blood volume and are in the bottom later, and

- A small, off-white region between plasma and red blood cells is called the **buffy coat** and is <1% of the total volume. It contains white blood cells and platelets.



Blood Sample Collection Procedures

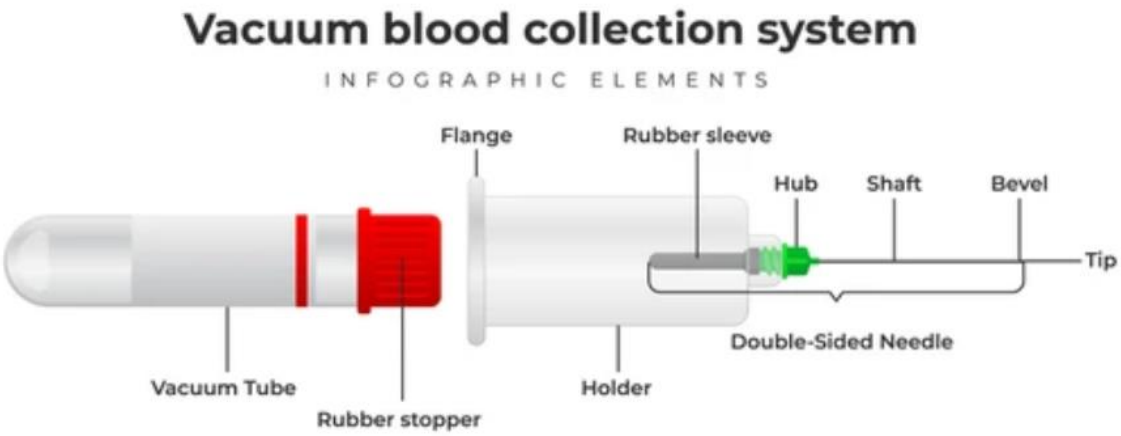
There are mainly in three ways of blood sample collection:

- **Arterial blood sampling**: Arterial blood sampling is a complicated process and should be done usually by trained personnel preferably by physicians only. Secondly, this type of sampling is not done at peripheral laboratories; hence is not discussed here.
- **Venous blood sampling**: Blood sample collection is also known as Phlebotomy. There is little difference between Phlebotomy vs. Venipuncture. Venipuncture is the process of puncturing a vein for different medical reasons. Medical professionals may use venipuncture to initiate intravenous therapy for extended periods or to collect blood samples by piercing the patient's skin.
- **Capillary blood/Finger-Prick Sampling:** Finger-Prick sampling, also known as Finger-Stick sampling, takes a small amount of blood from the patient to produce a micro-sample, usually from the fingertip. Finger-Prick sampling method is a quick method requiring very little preparation. It helps reduce stress and anxiety in patients, particularly children and nervous adults. It is commonly used at home by diabetic patients for their blood sugar level estimation using Glucometer and does not require a phlebotomist.

NOTE: Some special attention needs to be given during the collection of blood samples from Paediatric patients

Venepuncture blood Sampling:

It is the most commonly used procedure to collect blood from adults or patients. A needle takes blood from a vein, usually for laboratory testing. The blood sample is drawn from any of the upper limb's superficial veins, generally the median cubital vein in the arm. Since this vein does not have large nerves nearby, the procedure is significantly less painful and does not cause discomfort. The procedure is commonly performed by a trained phlebotomist, medical laboratory technician, or nurse inpatient OPD sample collection centers, wards, or the Clinical laboratory sample collection area. However, some patients find it inconvenient and stressful. There are chances of sample contamination also while sample collection is proper skin disinfection is not done with a proper disinfectant and with an appropriate time of contact.



Steps to follow before starting and during the Veni-Puncture procedure:

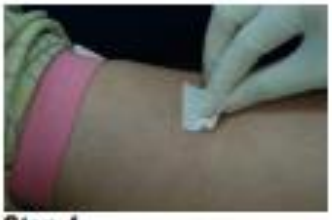
- Counseling of patient
- Vein selection,
- Decide the order of draw,
- Arrange material for test-specific handling, storage, and transportation requirements,
- Identify the anatomy of the antecubital area,
- Follow safety precautions,
- Identify alternative sites if your first choice fails etc.
- Label the sample collection vials as per test requirements
- Put the tourniquet on the patient's arm about 3-4 inches above the vein puncture site.
- Ask the patient to form a fist so that the veins become prominent.
- After finding the vein, clean the venepuncture area with 70% alcohol in a circular motion from inside to outside.
- Let the skin dry to have a proper contact period.
- Assemble the needle and vacuum tube holder.
- Insert the sample collection tube in the vacuum tube holder until it reaches the needle.
- Remove the cap from the needle of the vacuum tube holder.
- Press the skin about 1-2 inches below the needle insertion site with your thumb.
- Insert the needle into the vein and push the collection tube completely so that the inner needle penetrates the tube cap and blood starts pouring into the collection tube.
- The tourniquet should be released as soon as the blood is seen in the hub of the needle, to prevent hemoconcentration. After opening the patient's hand, place the spirit-soaked cotton swab on the puncture site, press it and ask the patient to keep it pressed for 3-5 minutes until the blood stops coming out of the venepuncture.
- Properly dispose of syringes, needles, and contaminants like cotton/gauge etc.

Venepuncture Procedure Photographic representation

Step 1
Label the tube with the patient's particulars

Step 2
Put tourniquet on the patient about 3-4' above the venipuncture site

Step 3
Ask patient to form a fist so veins are more prominent



Step 4
After finding the vein, clean the venipuncture site with alcohol using circular motion. Allow the area to dry

Step 5
Assemble needle and vacuum tube holder

Step 6
Insert the collection tube into the holder until the tube reaches the needle

Step 7
Remove cap from needle

Step 8
Use thumb to draw skin tight about 1-2" below the venipuncture site

Step 9
Hold the skin tight through step 10. Insert the needle, bevel side up, into the vein

Step 10
Push the tube completely onto the needle. Blood should begin to flow into the tube until vacuum tourniquet is exhausted

Step 11
Release the tourniquet

Step 12
After opening the patient's hand, place dry gauge over the venipuncture site and slowly remove the needle

Step 13
Apply mild pressure to the pad

Step 14
Apply bandage or continue applying mild pressure until bleeding has stopped

Step 15
Properly dispose of all contaminates supplies in sharp / biohazard container

Disposal of used syringes and needles: The syringe and needle should be destroyed in such a way as per the latest CPC rules regarding Bio-Medical Waste Management so that they should not be reused and not spread infections. The syringe needle destroyers shown in the figures below are mechanical and recommended. These instruments cut the syringe hub and needle into three pieces, leaving the syringe and needle unfit for re-use.

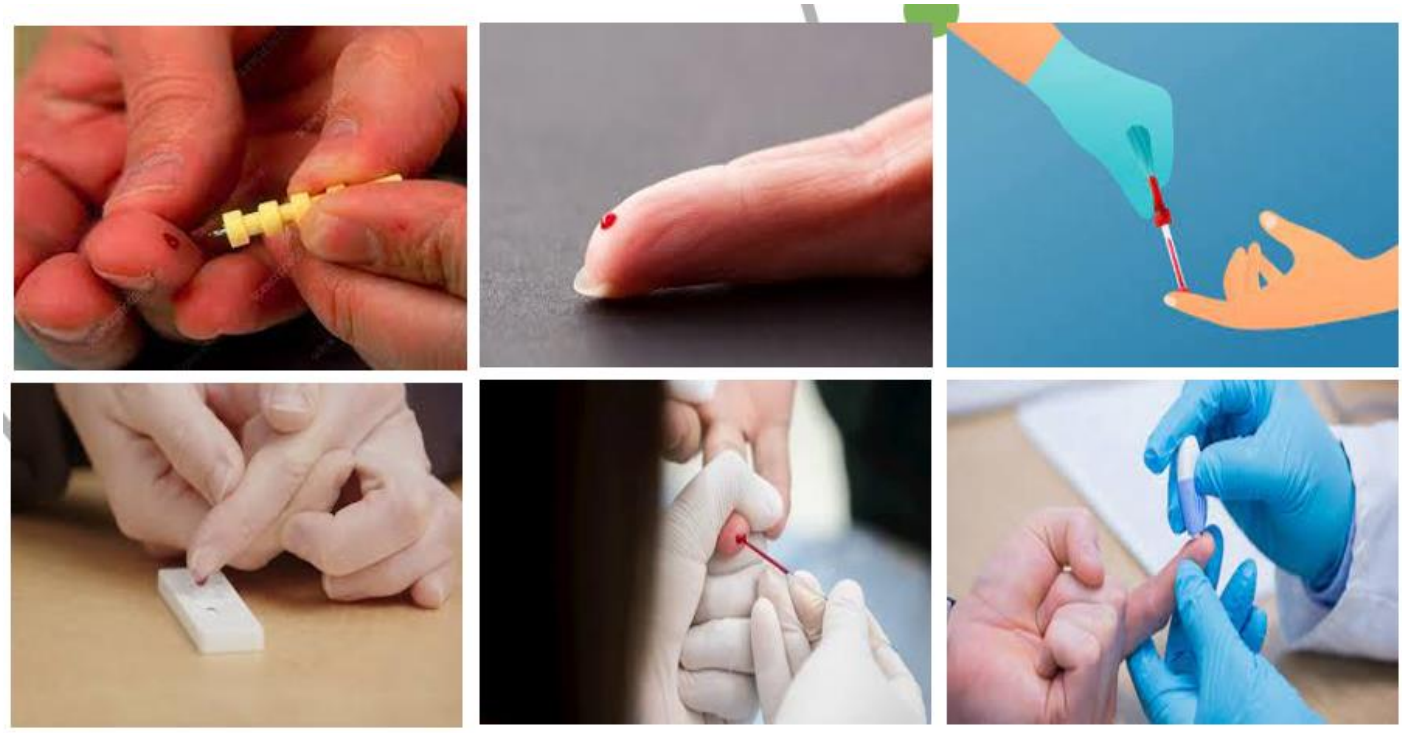
To deal with Biomedical waste management, refer to Biomedical Waste Management Section. However, in brief, for disposal of syringes and needles, follow the following procedure:

i. Never re-cap the needle with bare hands
ii. Use a one-hand scoop technique to recap the needle
iii. After thatput the needle in a white colored hard sharp needle disposal container
iv. Dispose of as per applicable Biomedical Waste Management Rules
v. This waste includes items like needles, syringes with attached needles, needles from devices that cut or burn needle tips, scalpels, blades, and any other sharp object that is contaminated and can potentially cause punctures or cuts.
vi. Collect the waste in a white translucent, puncture-proof, leak-proof, tamper-proof container.
vii. **Treatment and Disposal**: For Health Care Facility **(HCF)** having linkage with Common Bio-medical Waste Treatment and Disposal Facility **(CBWTF)**: After collection in a puncture-proof, leak-proof, tamper-proof container, handover the waste to CBWTF without any alteration or onsite treatment.

Finger-Prick Sampling:

This method is considered the best way to collect a blood sample where ever possible Keeping in view the patient's comfort and welfare at the point of collection. Only a few drops of blood are taken to produce a

"micro-sample" using this type of sampling; the long-term benefits of this methodology include less loss of blood and the ability to carry out testing at home.



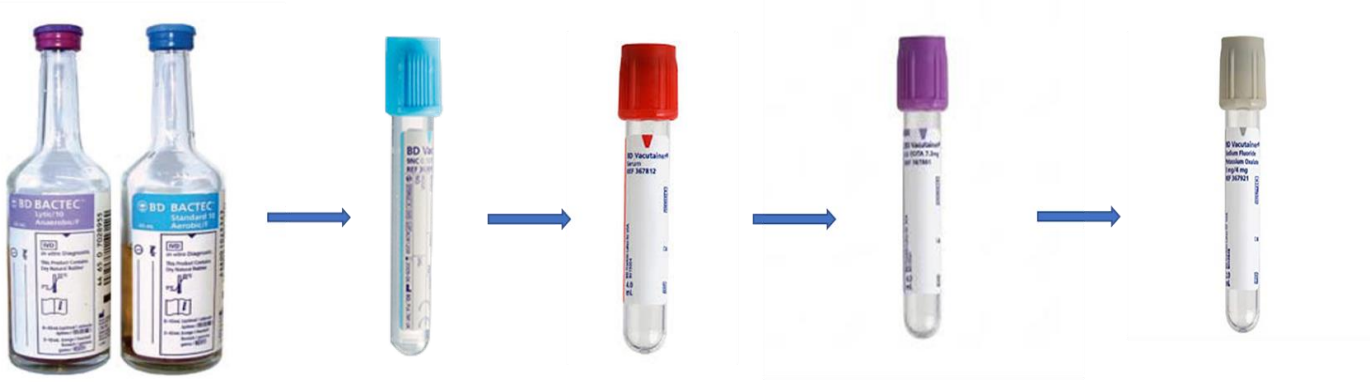
Procedure for Finger Prick Sampling

For adult Patients:

- Apply alcohol to the entry site at the fingertip and allow it to air dry.
- Puncture the skin with one quick, continuous, deliberate stroke to achieve good blood flow.
- Wipe away the first drop of blood because it may be contaminated.
- Avoid squeezing the finger too tightly because this dilutes the specimen with tissue fluid (plasma) and increases the probability of hemolysis.
- After the blood collection procedure is completed, apply little pressure to the puncture site to stop the bleeding.

Take laboratory samples in the correct order:

- All Microbiology Samples
- All Hematology samples
- All Biochemistry samples and
- All Blood banking samples



Sampling from Paediatric and neonatal patient

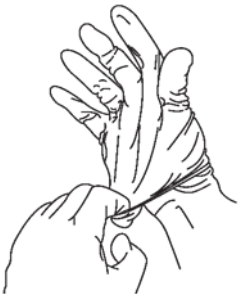
Taking blood samples from a child is difficult. This should be done carefully after putting the child at ease without creating anxiety.

A general illustration for Capillary blood sampling

1. Lancet and collection tube.

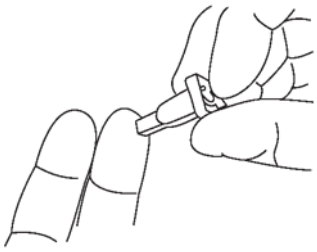
2. Assemble equipment and supplies.

3. Perform hand hygiene (if using soap and water, dry hands with single-use towels).

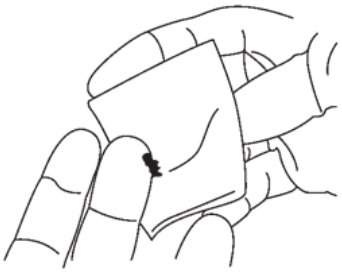


4. Put on well-fitting, non-sterile gloves.

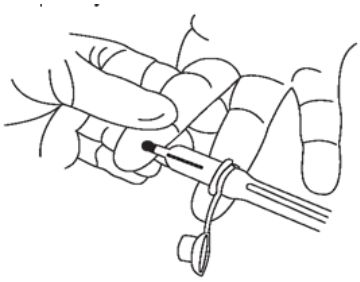
5. Select the site. Apply 70% isopropyl alcohol and allow to air dry.



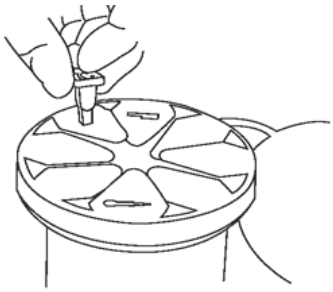
6. Puncture the skin.



7. Wipe away the first drop of blood.

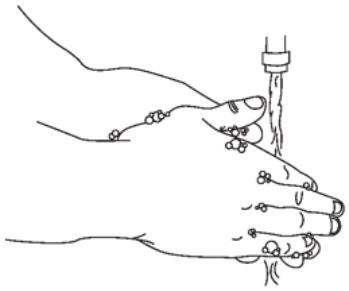


8. Avoid squeezing the finger too tightly.



9. Dispose of all sharps appropriately.

10. Dispose of waste materials appropriately.



11. Remove gloves and place in general waste. Perform hand hygiene (if using soap and water, dry hands with single-use towels).

Sample Transportation:

After collection, the samples should be transported to the laboratory and processed as soon as possible because delay may lead to some changes which may affect the sample test result adversely, i.e., If the blood sample is delayed, it may lead to the following adverse changes:

- Glycolysis (Conversion of Glucose to lactate) may occur.
- Plasma inorganic phosphate levels may increase.
- Nitrogenous substances may convert to ammonia.
- Pyruvate may be converted into lactate.
- Carbon dioxide may loss

Urine samples should be kept/preserved in cool conditions (2-8°C) as urine samples may contain some bacteria in low numbers which are mixed in it from the urethral front. If kept at ambient temperature, they keep multiplying, leading to many changes in urine samples, possibly leading to a wrong conclusion.

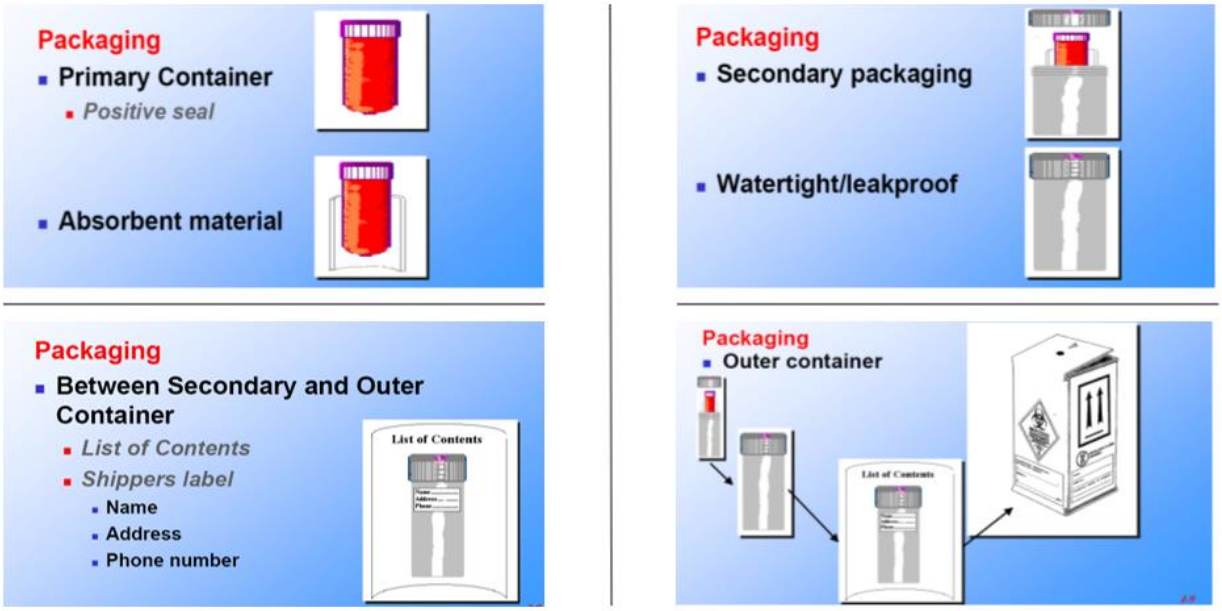
CSF or any other sterile body fluid samples, particularly for bacterial/fungal culture, should be preserved at room temperature, not in cold conditions. The CSF sample for cell counting and cytological examination

should be preferably processed immediately upon receipt, in order to prevent the degenrtaive changes in the cellular morphology and counts. If the delay is inevitable, the sample can be kept in refrigerator at 4^{O} C. If required, the sample should be transported in a closed, leakproof box/container as soon as possible. Insulated containers are preferred to avoid exposure to light and high environmental temperatures.

The MLT should also be able to pack the samples safely and transport clinical samples to higher competent laboratories for advanced testing or any other purpose, keeping in view all sample and environmental safety requirements particularly transported by air/train etc., to a distant laboratory.

Inadequate closing and packing of samples

i. Contaminate wrappings and forms/documents/ packing inserts
ii. Outside of samples – may be contaminated and should be treated as potentially hazardous at receiving in the lab.



Receipt of Samples in the laboratory:

Usually, the clinical samples are received in the laboratory in two ways:

- Samples were collected in a sample collection center away from the testing laboratory
- Sample collected directly in the laboratory

In both cases, proper labeling and preservation conditions are essential. But when the sample is collected in a sample collection center away from the testing laboratory, it becomes vital to

a. maintain sample accession record
b. check the sample condition on receipt in the testing laboratory, particularly about the transport conditions and other sample suitability conditions like a blood sample is not hemolyzed and s sputum sample is a proper sputum sample and not saliva only.

After checking all suitability conditions, the samples are given sample ID, or if it is labeled with a bar code at the sample collection center, then the same bar code should be considered as its sample ID. Sample receipt time record is important in both cases to calculate turnaround time (TAT)

After collection/receipt of the sample, a competent MLT shall be able to know the procedures and importance of chain of custody relating to specimens as per requirements of the test procedure:

A chain of custody (COC) is a set of traceable records that provide unbroken control over a document, raw data, or a sample and its containers from initial collection to final disposal.

Laboratory-based chain of custody strives to answer the following questions:

- "Where is my sample now?"
- "Who possesses my sample now?"
- "When did he/she take possession of my sample?"
- "Where has my sample been?"
- "Who has been in possession of my sample?"

Using a properly recorded Chain of custody, one may find out the delay, if any, at any point of processing. It also helps the analysis of TAT. The laboratory personnel should know where the sample is at a given time and in whose custody it is.

The bigger and established laboratories use the electronic Laboratory information system with barcoding. But the smaller laboratories still use manual systems to establish the chain of custody. For that, a competent MLT shall:

- Label and verify the sample container properly with the patient name and ID
- At the sample collection area, he shall enter the details of the sample into the permanent sample entry register and also shall enter in sample accession record format to be handed over to sample transporters.
- Reaching the laboratory, the recipient MLT shall verify the sample details, match them with the sample accession record and sign the sample accession record that he has taken the sample into his custody.
- The sample will now be entered into the laboratory record register and put into the test process.
- After generating the report, it is entered into the laboratory register
- Before release, the report is verified/validated by senior and authorized personnel

- On releasing the report, again, it should be mentioned in the laboratory record that the report has been released and also to whom the report is handed over
- After releasing the report, the sample is now retained for a time, as mentioned in SOP, and is preserved under suitable conditions maintaining its integrity till it is disposed of. At this level also, it is recorded in retained sample record register
- After completion of the recommended retaining period, the sample is disposed of as per Biomedical Waste management rules, and the details are mentioned in the records regarding its final disposal, including who and how it was disposed of.

The test result greatly depends upon the pre-analytical phase activities. Hence after going through this domain, the trainee will learn the following:

Lessons Learnt

- To verify relevant information provided for test requests on requisition form mainly, which is essential for the interpretation of results or critical alerts or recording purposes.
- To assist/work with Common Laboratory Procedures.
- To confirm the patient's identity and perform venipuncture and capillary blood collection to obtain appropriate samples for laboratory analysis.
- To provide all relevant information about precautions on specimen collection, transportation, and storage To the patients/clients wherever required, like "how to collect a urine sample for microbial culture from a male or a female patient?"
- To perform patient counseling/preparation for collection of clinical samples
- to demonstrate the types and use of procedures or tools for sample collection like vacutainers for blood samples
- to demonstrate what type of sample is to be collected for a required test like serum plasma or whole blood etc**.**
- If required, understand the types of anticoagulants and their uses for blood or other samples.
- To understand various types of sample containers/swabs used as per the requirement of the test, e.g., Sterile containers /swabs are used for microbial culture samples.
- To demonstrate skin disinfection procedures before taking any blood sample or wherever required.

- To perform the collection of samples for tests routinely done at PHCs and chain of custody procedures relating to specimens as per requirements of the test procedure.
- To adhere to established protocols for labeling and traceability of specimens
- To identify and process specimens taking into account priority and sample stability
- To assess the suitability of the specimen for testing
- To verify that the pertinent data on the specimen and requisition correspond.
- To access specimens into laboratory information systems/hard copies or registers.
- Adheres to specimen retention, storage, transportation, and disposal guidelines.
- To identify, document, and initiates corrective action for pre-examination (pre-analytical) errors.
- To prepare blood, body fluids, and other clinical specimens for microscopic examination

Analytical Phase:

Use of Validated Methods

Only those test procedures should be used in examination processes that have been validated for their intended use to assure the clinical accuracy of the examination for clinical sample testing. Two types of methods are mainly used in clinical laboratories:

- **In-house developed and standardized/validated methods** include all the manual methods, i.e., Haemoglobin testing by Sahli's and staining methods. In this case, the reagent is prepared by lab personnel in laboratory environmental conditions; hence may not be as efficient as commercially available reagents. So, such reagents/procedures should always be validated in the laboratory using standard reference controls. Once standardized/validated test procedure or reagent should not be changed until a new procedure/reagent is validated. The validated methods should be displayed on workbenches as SOPs which all personnel should follow without skipping any step and following all time intervals between test procedure steps.
- **Commercially available and manufacturer-validated test procedures:** Almost 95% of test procedures and materials are available commercially, including test kits like card tests and other diagnostic test kits. Suppose these kits are available with some automated equipment, then usually, calibration of equipment and validation of kits or reagents are internal and automated in the equipment. Company engineers do major calibrations.

Test/equipment SOPs following and understanding their importance: Standard Operative Procedures (SOPs) are the written document that comprises the stepwise procedure to perform a process/method or to operate some equipment. Along with the step-wise procedure, it also includes the working principle, materials required, quality control procedures, reference material, reference documents, and precautions while performing a process or using the equipment.

For every test procedure or use of equipment, an independent SOP should be prepared and displayed on the workbench or at the place of equipment so that while performing the procedure or using equipment, the worker should see that and work accordingly.

Why SOPs are required:

- Standard Operating Procedures are required as:the personnel may become over-confident and try to set the steps or step timings according to his convenience without the validation of new steps
- may skip some steps considering those as insignificant, which may affect the results of the test

- If one person is on leave and the second person does not remember the procedural steps, either they won't perform the test or will do the test with less efficacy.

Therefore it is important to have written down SOPs, train the MLTs on it and have it displayed in the workplace.

Able to assess results, identify sources of interference, and initiate corrective action

A competent MLT is knowledgeable and skilled in assessing the results, matching those with control results, and finding/detecting any irregularity. If any irregularity is detected, he/she should know the possible causes and confirm the cause of the current error. After finding the cause of irregularity, he/she will initiate corrective action to eliminate the cause of the abnormality.

For example:

The air pressure surrounding the flame, the oxygen concentration, and the presence of oxides like Carbon dioxide, Nitrogen dioxide, and Sulphur dioxide in the surrounding air interfere with the results of the flame photometer.

1. **Biological reference intervals and clinical decision limits**

- Trainers and district/state authorities should ensure that biological reference intervals and clinical decision limits are appropriate for the catchment population to which the the laboratory caters.
- The biological reference intervals and clinical decision limits should be reviewed on a regular basis, and any changes made should be properly conveyed to the users.
- Suppose there are any alterations to examination or pre-examination methods, the district/state laboratory should review any potential impact on associated biological reference intervals and clinical decision limits. If necessary, any relevant changes should be communicated to the users.

Internal quality control (IQC) and all the tests or test batches should be included.

Performing Card-based immunoassays tests and tests using commercially available ready-to-use kits for tests done at PHCs with knowledge of all the precautions affecting the test results

Card or strip-based tests, called Rapid tests or rapid diagnostic tests (RDTs), are simple and convenient tests that yield rapid results, typically within 30 minutes or less. These tests are user-friendly and require minimal effort to conduct. Rapid tests are primarily utilized for the diagnosis of infectious diseases, such as:

- Flu
- Strep throat

- Malaria
- HIV
- Dengue
- COVID 19

Rapid and easy tests are also used in certain home-based tests, such as pregnancy tests and blood glucose level testing. These tests should be performed only after thoroughly reading and understanding all the procedures and instructions from the pack insert.

Precautions while using commercial kits:

- Check that the kit is adequately preserved under the required storage conditions mentioned on the kit, e.g., 2-8°. If in the refrigerator, the kit should not be wet, or its printing should not be spoiled as it occurs only if the refrigerator did not work for a significant time and the cold chain has been broken.
- Check the expiry date
- Open the kit, take out the package insert, and read the instructions for use and methodology, including the validation process. Match all these parameters with existing SOPs. If there is variation, the SOP has to be changes as per kit insert.

Benefits of Rapid tests include:

- Quick Results: This could enable the patient to receive treatment promptly
- User-friendly: Rapid tests can be conducted by non-medical personnel and trained volunteers. In some cases, even self testing is possible.
- Minimal equipment needed: These tests do not require specialized equipment, making them particularly advantageous in regions with limited resources or access to specialized laboratories.

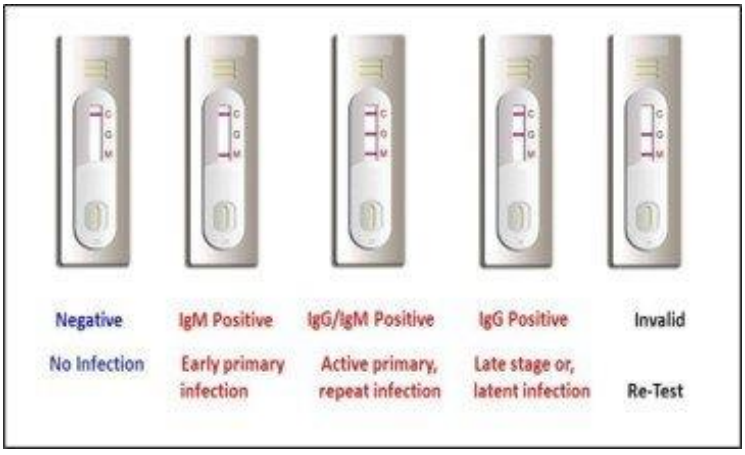
Drawbacks of Rapid tests:

- Rapid tests have lower sensitivity compared to lab tests. Sensitivity refers to the ability of a test to accurately detect a disease or condition. Rapid tests are not as effective as lab tests in identifying diseases during the early stages of infection.
- False negatives are more frequent. A false negative result means that the test indicates a person does not have a disease or condition when they do.

Important Example

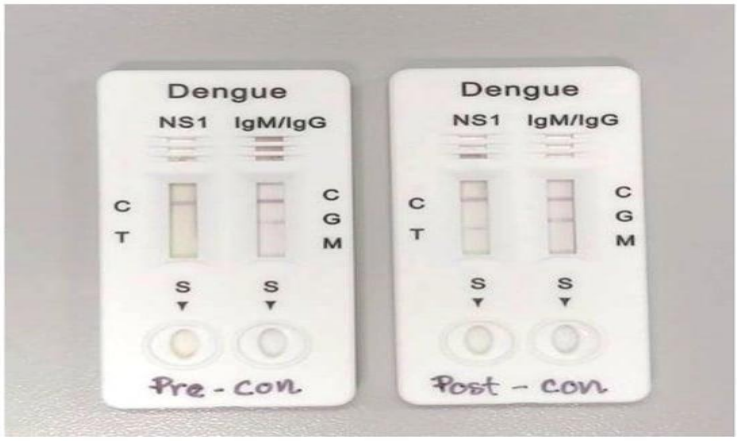
Typhidot card test is a ready-to-use dot ELISA kit designed for qualitative detection and differentiation of these antibodies separately against the outer membrane protein (OMP) of the Salmonella typhi bacterium.

It detects both IgG and IgM antibodies against Salmonella typhi. The photograph shows the cards with different results as per the infection duration. IgM positivity shows the recent infection, but if IgM is negative and IgG is positive, it indicates late infection.



Dengue Combo (NS1 + IgG/IgM) Rapid Test

The Dengue Combo Test is a quick and efficient test that utilizes solid-phase immuno-chromatography. Its purpose is to detect the presence of Dengue NS1 Antigen and differentiate between IgM and IgG antibodies to the Dengue virus in human serum or plasma.



Able to apply principles of commonly used analyzers/ semi-auto analyzers for hematology and biochemistry if available at respective PHC

An automated analyzer is a laboratory device created to rapidly assess various chemicals and other characteristics in multiple biological samples with minimal human intervention. By analyzing the properties of blood and other fluids, these measurements can provide valuable insights for disease diagnosis.

These days many tests are being performed on autoanalyzer, which may be semi-auto or fully autoanalyzer for Biochemistry, hematology, endocrinology etc.

To increase the competency of an MLT, he/she should be given proper training on its functioning, interferences, quality controls, calibrations (Internal and External), Quality control reference material, and troubleshooting by the company engineer at the time of installation. Different labs have different analyzers, so MLTs should be trained only by Company engineers.

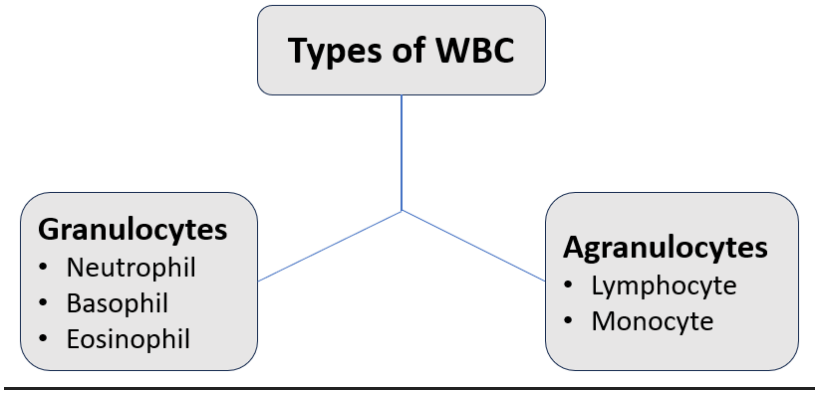
Performing manual cell counting procedures using microscope and cell counters etc.

Some tests in clinical laboratories are performed manually even today, like Total leucocyte count (TLC) and Differential Leucocyte Count (DLC) using Neubauer Counting Chamber and stained smears, respectively. The MLTs should be capable of performing these tests manually with reference methods. They should have knowledge of the Principle of the test, Material required, working principle, test procedure, recording the observations and calculations of the test result.

WBC(Leukocyte)/TLC Manual Counting

The WBC or leukocyte count method measures the number of white blood cells in a microliter of blood. By determining the total count or concentration of leukocytes, the immune status can be assessed.

When there is an increase in leukocyte concentration, it is known as "Leukocytosis,"and a decrease in leukocyte concentration leads to "Leukopenia." Therefore, white blood cells are crucial in maintaining overall health, as immune function depends on them.



- Neutrophils have a segmented nucleus and contain light pink granules in the cytoplasm. They are important for engulfing bacteria and promoting inflammation.
- Basophils have a bilobed nucleus and dark blue or purple granules in the cytoplasm. They are involved in allergic responses.
- Eosinophils have a bilobed nucleus and contain dark pink or red granules in the cytoplasm. They are effective against parasites and helminths.
- Lymphocytes have a large rounded nucleus with a cytoplasm that does not contain granules. They include T, B, and NK cells that initiate immune responses.
- Monocytes have a large, indented nucleus with a cytoplasm that does not contain granules. They play a key role in forming macrophages and other antigen-presenting structures.

Principle: Test blood sample is mixed and diluted with a weak concentration of Acetic Acid (In specified known volume). Weak acids will lyse red blood cells and darken WBCs to facilitate counting by the hemocytometer. Manual WBC counting is used in cases of very low WBC count (Leukopenia) with automated Hematology Cell Counters and when automated cell counters are unavailable.

Sample: EDTA anticoagulated whole blood.

Reagents and supplies required:

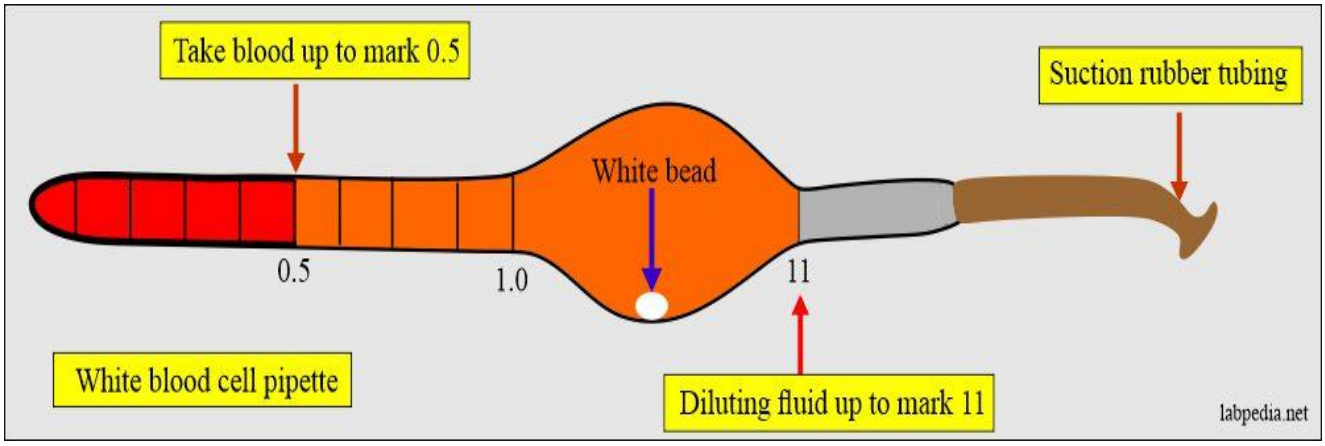
- Volumetric Flask 100 cc.
- Serological pipettes or micropipettes.
- Concentrated Acetic Acid (Glacial acetic acid)
- Distilled water or Deionized water.
- Diluting fluid

Preparation of Diluting fluid:

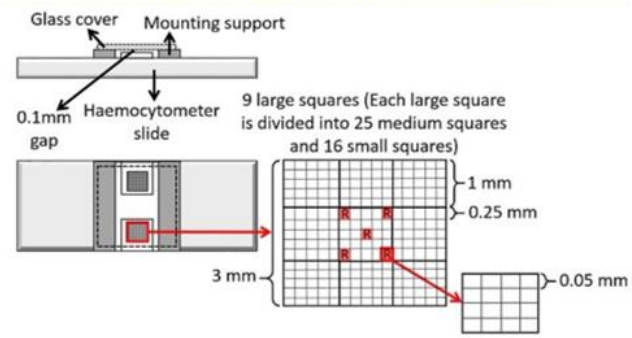
2 % Acetic Acid in distilled water (Turk's solution) -2 ml glacial acetic acid +98 ml Distilled water.

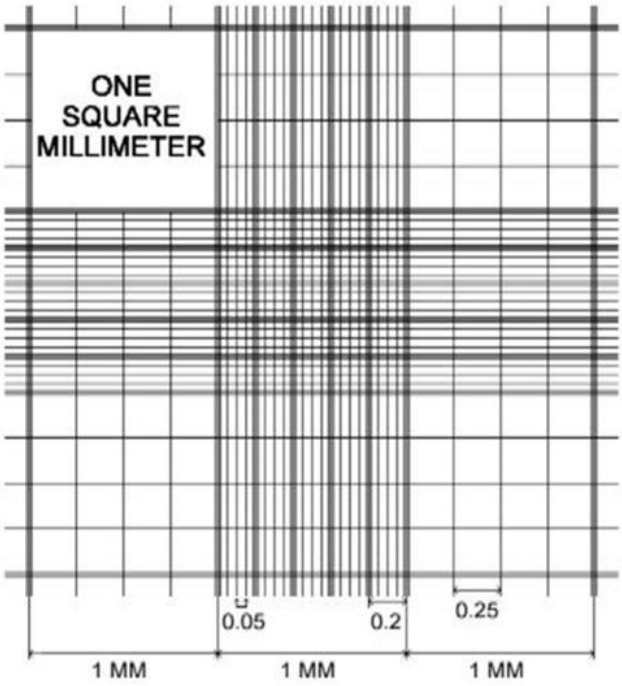
Glassware, Apparatus, and Equipment Required :

- WBC/TLC/Thoma pipette: This pipette, also called the 'Thoma' pipette (As shown Below), has a long stem that is divided into two parts:
    - The extended part of the apparatus is labeled with 0.5 and 1.0 markings.
    - The shorter arm following the bulb is marked with "11".
    - The central section of the apparatus is a bulb-shaped container with a single white bead inside.
    - A rubber tube is connected to extract the blood.
    - The final blood dilution to the TLC fluid is 1:20.



Improved Neubauer Counting Chamber





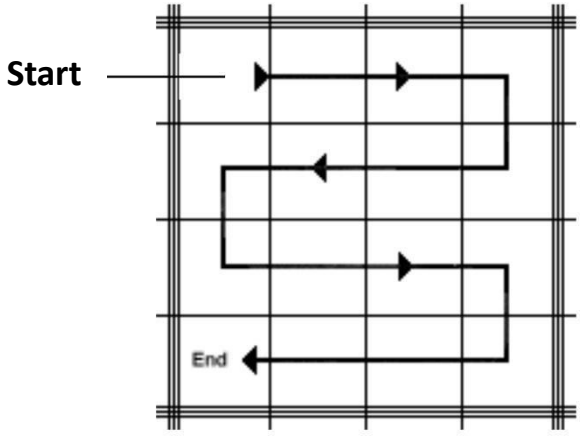
- Clean cover slip slide (specially made for the Hemocytometer)
- Automatic micropipette to deliver 20 microliters and 380 microlitres
- Gauze 10 x10 cm
- Glass / Plastic tubes (12 x75 mm)
- Handy tally counter
- Conventional light microscope.

Procedure:

- **Total leucocytes count (WBC) with Thoma pipette method:**
    - Fill the blood into TLC pipette up to the 0.5 marks, and then the TLC solution is added to point 11.
    - Remove the rubber tubing and seal both ends or hold between two fingers of gloved hands.
    - Better is to put this pipette on the mechanical device to shake it for 1 minute, preferably 2 minutes, to make a homogeneous mixture of Leucocytes in solution.
    - After thoroughly mixing, discard the first few drops and gently fill the chamber to fill the platform.
    - The capillary action will draw the fluid.
    - Allow the chamber on the microscope stage for 2 to 3 minutes till the cells are settled.

In case the TLC tube is not available, one may proceed with the tube method, which is as follows:

- **Test Tube method:**
  - Mix the blood sample gently but thoroughly by inversion 5-8 times. (or by mechanical rocking mixer)
  - Pipette 0.38 ml (380 microlitres) of diluting fluid into a 12x75 mm tube.
  - Pipette 0.02 ml (20 microlitres) of well-mixed blood to be counted and wipe the tip with gauze into the tube containing diluting fluid and mix the tube.
  - Let the tube stand for 2-3 minutes to ensure complete RBC lyses, then mix well.
  - Prepare the clean Haemocytometer and cover it with the designed coverslip.
  - Load one side of the Haemocytometer with a capillary tube or micropipette, do not attempt to overload or underload the hemocytometer.
  - Allow the Haemocytometer to let sit for several minutes to allow the WBCs to settle in the counting chamber, to avoid a drying effect, place the loaded Haemocytometer in a covered Petri dish with moist gauze until counting.
  - Place the Haemocytometer in the microscope stage.
  - Focus with an x10 objective lens (low power) by lowering the condenser.
  - The WBCs are counted in the four counter large squares with a hand tally counter.
  - Follow the counting pattern shown in the figure below. During counting, do not count cells that touch the right or bottom boundaries to ensure unduplicated counting.



  - The total counted WBCs in the four large squares are added together.
  - Suppose the number of cells in a square varies from any other square by more than nine cells. In that case, the count must be repeated because this represents an uneven distribution of cells, which may be caused by improper mixing of the dilution or improperly filled hemocytometer.

- **Calculation: Total WBC count**
  - N (Number of cells counted in 4 squares) x DF (dilution factor:20)
  - Area counted (4 mm squares) x depth of fluid (0.1 mm)
  - Example:

    50 49 48 47

    52 49 56 46

    43 48 52 51

    50 51 49 46

  Corner four squares where cells are counted in 16 squares in every corner, one corner square

$$\text{Total Leucocyte Count} = \frac{787 \text{ x4 x } 20}{4 \text{ x } 0.1} = 157400/\text{cmm or microliter}$$

Reference Range:

- Adults: 4500-11000/cumm
- Six years: 4500-12000/cumm
- One year: 6000-14000/cumm
- Newborn: 9000-30000/cumm
- WBC count varies according to age but not to sex.

Sources of error:

1. Contaminated or diluted fluid.
2. Incorrect dilution.

Differential Leucocyte Count (Manual Microscopy method):

Principle: Differential count is the relative portion of white blood cells when stained with Romanowsky stains and counted in an oil immersion lens and out of 100 cells with the percentage given.

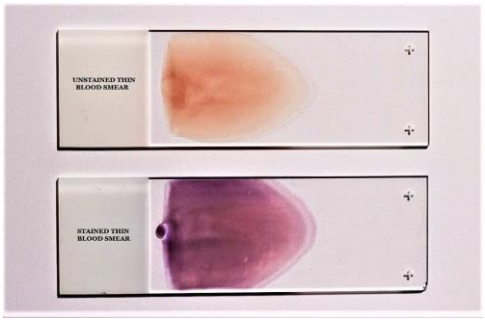
Material required:

- Leishman stain
- Sorensen phosphate buffer with pH6.8
- Staining rack in horizontal position.
- Pasteur pipette
- Immersion oil with RI 1.50-1.52
- Microscope (with x10 and x100 objective)

Procedure:

- After staining of Blood smear with Leishman stain,

  Smears are air-dried.



Leishman Stained and Unstained Blood Smear

- Slides are placed on the microscope stage.
- Adjust the stage and focus with x10 objectives.
- Focus on the ideal counting area (tail and pre-tail portion of film)
- Shift the objective to x100 and put immersion oil and fine adjust to focus the smear with the condenser upside position.
- Choose areas where RBCs are side by side and not overlapping.
- Make a sheet of 10 x10 squares (as shown in the recording of the results) for the exact counting of morphologically identified cells.

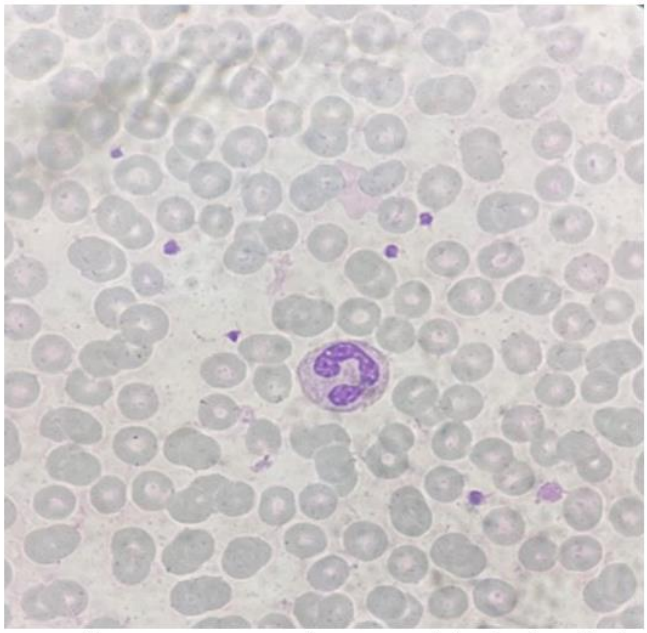
Normal Blood Smear Stained with Leishman Stain Under The Microscope

Quality Control in Leishman's Stain: Internal quality control of Leishman's stain must be performed regularly on known reference material. Erythrocytes stain yellowish red. Lymphocyte nuclei stain deep purple; cytoplasm of lymphocytes stain light blue. Eosinophil nuclei blue, red to orange granules, blue cytoplasm.

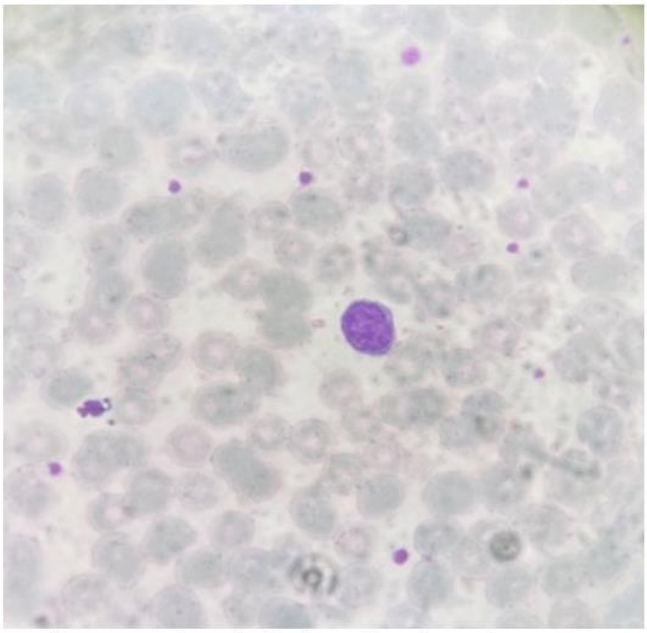
Colours as shown by different components after staining blood film with Leishman's stain while evaluating a normal quality control smear under the microscope:

Sr No	Cell/component	Colour
1.	Chromatin	Purple
2.	Nucleoli	Light blue
3.	Erythrocytes	Light pink
4.	Reticulocytes (Cytoplasm)	Dark blue
5.	Lymphocytes (Cytoplasm)	Blue
6.	Monocytes (Cytoplasm)	Grey blue
7.	Neutrophils (Cytoplasm)	Bluish pink
8.	Neutrophil (Granules)	Purple
9.	Basophils (Cytoplasm)	Blue
10.	Basophil (Granules)	Purple black
11.	Platelets (Granules)	Purple

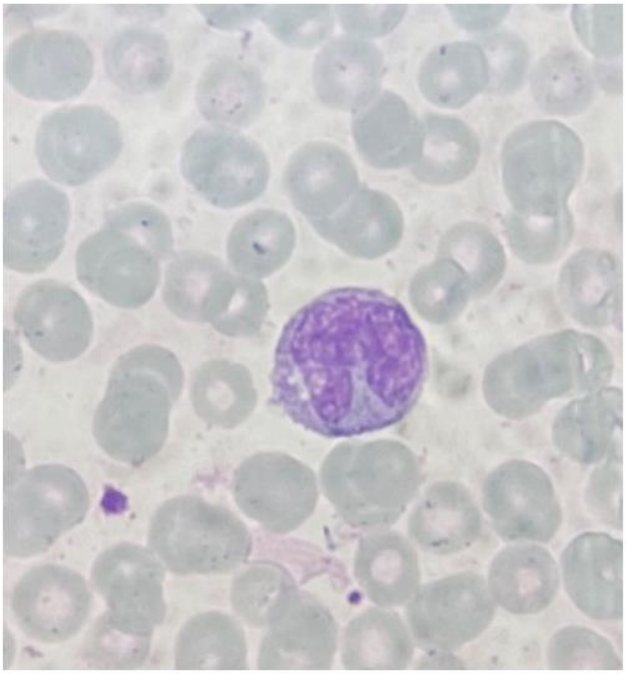
Morphological identification:



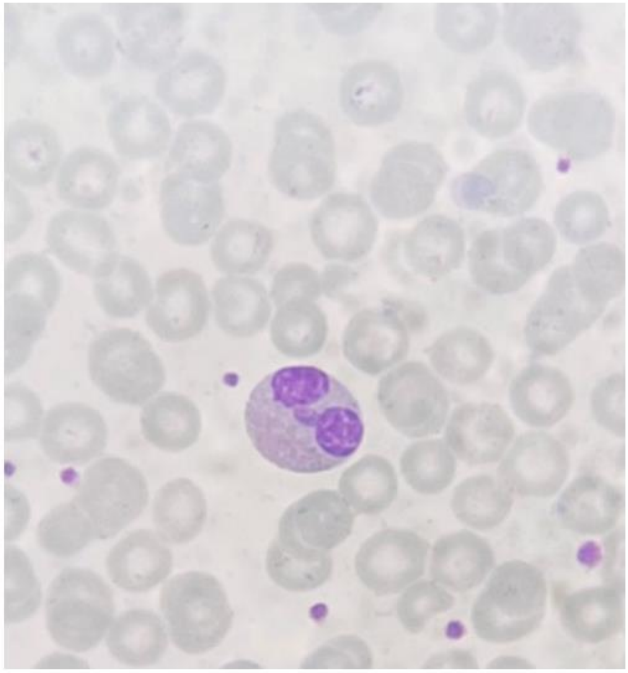
Neutrophils : Size : 12-15 micron in diameter, Round shaped with 3-5 lobed nucleus , cytoplasm with azurophilic granules.



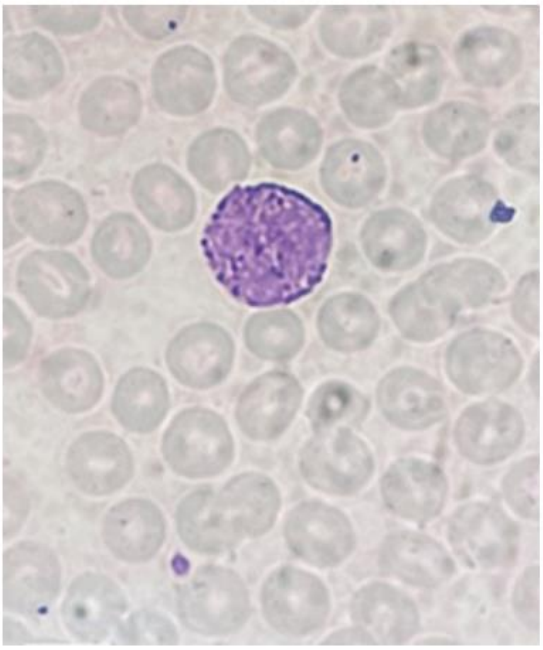
Lymphocyte : Round to oval with pale rim of cytoplasm with 10-12micron size and agranular cytoplasm .



Monocyte : 16-20micron cell with horseshoe shaped nucleus and ground glass cytoplasm with no or fine azurophilic granules. Cytoplasmic membrane oval to irregular in outline .

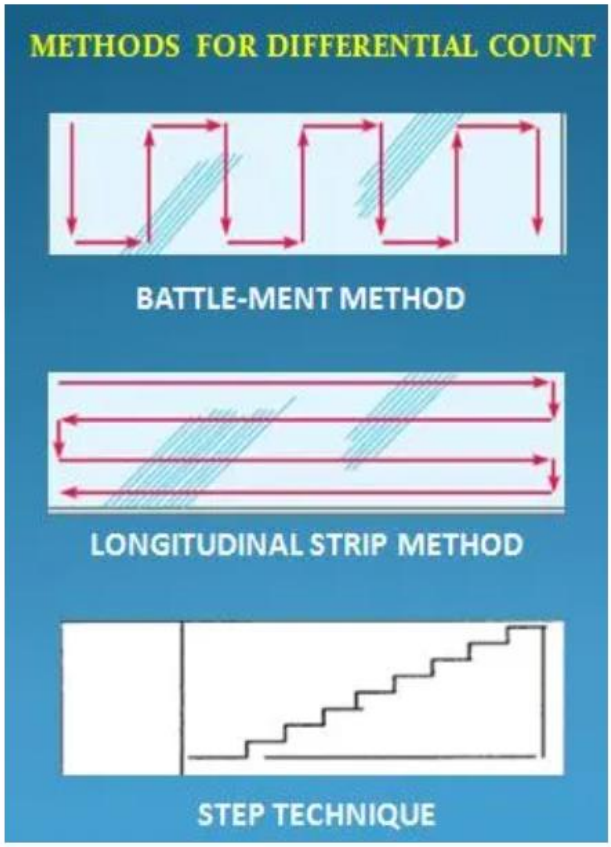


Eosinophil : 10-13micron size, coarse eosinophilic granules pinkish in colour with bi-lobed nucleus.



Basophil : 12-15 micrometre in size with coarse dark blue to purple coloured granules obscure the cell nucleus.

- Cells are counted by Battle-ment method and counted unless 100 cells are reached.



- **Recording the Results:**

  Different cells are recorded in the table below for the exact percentage of each and every cell.

N	L	N	N	N	E	L	L	L	N
N	N	N	N	N	N	L	L	L	E
N	N	N	N	L	L	L	N	L	E
M	N	L	E	L	N	N	N	N	E
N	N	N	N	L	L	L	L	N	E
M	B	E	E	E	L	N	N	N	N
N	N	N	N	N	N	N	L	L	E
M	M	N	N	N	N	N	N	N	L
N	L	L	L	L	N	N	N	N	N
N	N	L	L	N	N	N	N	N	N

Result of differential count (On the basis of the above table) :

- Neutrophil : 60 %
- Lymphocyte:26 %
- Monocyte : 4 %
- Eosinophil :9%
- Basophil : 1 %.

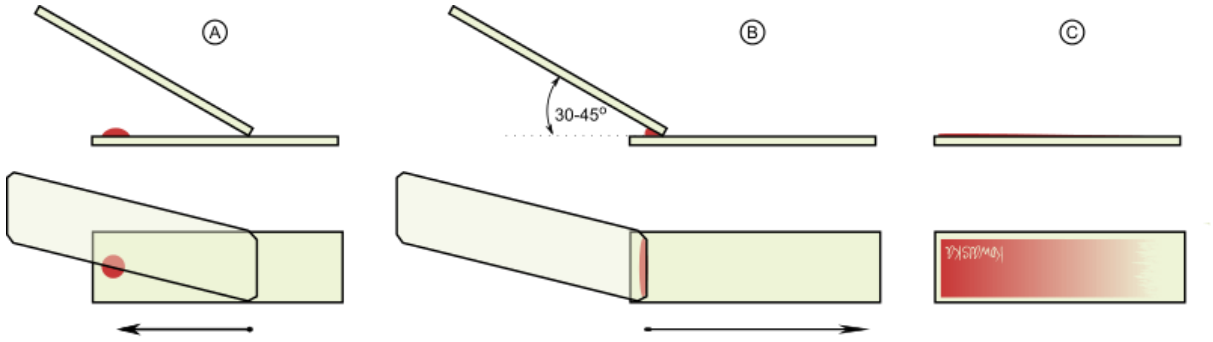
Able to Identify and evaluate the morphology of cellular and non-cellular elements in microscopic preparations

The MLTs should be competent in recognizing various cells and microbes in blood, urine, or other body fluid like CSF.

Microscopy:

Five major staining procedures are done at the PHC level

- **Preparation of Blood smears:** Mainly two types of smears are prepared, i.e., Thin smear and Thick smear
    - **Thin smear**: The main purpose of preparing thin smears is to examine blood cell counts and morphology and detect any blood parasites. Thin smears are created by spreading a layer of blood on a slide, with the thickness decreasing towards the feathered edge. The cells in the feathered edge must be spread out in a monolayer, not touching each other. Making at least 2 smears per patient is recommended when preparing thin smears.
    - Procedure for preparation of a thin smear of a blood sample
        - Apply a small droplet of blood onto the slide that has been pre-cleaned and labeled near its frosted end.
        - Bring another slide at a 30-45° angle and touch it to the blood droplet, allowing the droplet to spread along the line where the two slides make contact.
        - Swiftly push the upper slide with a smooth edge (known as the spreader) toward the unfrosted end of the lower slide.
        - Ensure that the smears have a well-defined feathered edge by using the appropriate amount of blood and employing the correct spreading technique.
        - Allow the thin smears to dry. They dry more quickly than the thicker smears and are less likely to detach from the slide because they will be fixed.



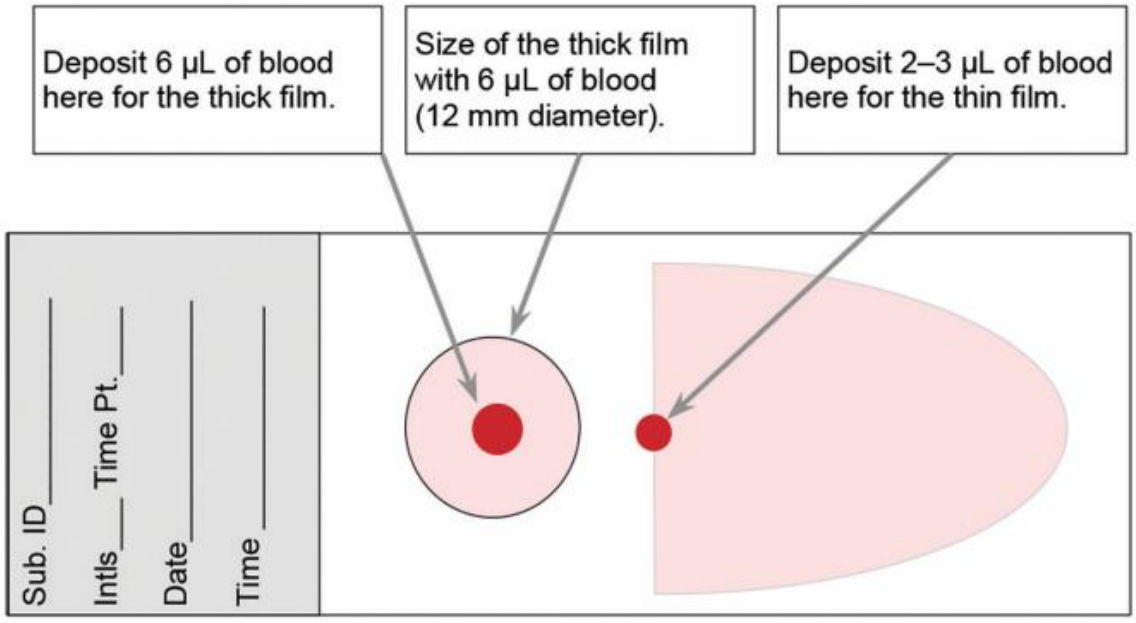
- **Thick smear**: Thick smear is prepared to detect blood parasites when they are expected to be present in low numbers.

  Thick smears are composed of a dense layer of dehemoglobinized (lysed) red blood cells (RBCs). The concentration of blood elements, including parasites, if present, is higher (approximately 30 times) compared to the same area of a thin smear. This makes thick smears more effective in detecting parasites, as they have increased sensitivity. However, thick smears are not ideal for accurately examining parasite morphology. For instance, they may not provide sufficient information for identifying the species of malaria parasites. If a thick smear shows positive results for malaria parasites, a thin smear should be used for species identification.

  Prepare at least two smears per patient!

  - Apply a small droplet of blood in the middle of a slide that has been pre-cleaned and labeled.
  - Using the edge of another slide or an applicator stick, spread the droplet in a circular shape until it measures about 1.5 cm2, similar to the size of a dime.
  - A proper density for the thick smear is achieved when one can barely read the text if placed damp over the newsprint.
  - Ensure the slides are placed flat and allow the smears to dry completely (protect them from dust and insects). Smears that haven't dried sufficiently and those that are too thick may separate from the slides during staining, particularly in those made with anticoagulated blood. At room temperature, drying can take many hours, with a recommended minimum period of 30 minutes. In this case, the smear must be handled with extreme care during staining. You may use a fan or hairdryer with a cool setting to quicken the process of drying. Avoid exposing thick smears to hot conditions since it may cause heat-fixing of the smear.
  - Thick smears should not be fixed by methanol or heat. If staining of the smears is delayed, the thick smear should be briefly dipped in water to haemolyse the RBCs.

Comparison of Thin and Thick smears for Malarial Parasite



Fixation of smears:

Dipping them in absolute methanol when using Giemsa stain to fix the smears is necessary. However, Leishman's stain already contains methanol that fixes the smear onto the glass slide, eliminating the requirement of separately fixing it with methanol.

Note: In situations where slides are limited, national malaria programs (as well as CDC personnel) may prepare a thick and thin smear on a single slide, particularly in field settings. This method is satisfactory, provided that only the thin smear is fixed.

Commonly used Staining procedures for blood films:

Leishman's staining

Principle: The stain contains both acidic and basic dyes that bind with blood cells' basic and acidic components, respectively. Eosin, an acidic dye, inconsistently stains the basic structures of cells, such as the cytoplasm and granules. On the other hand, methylene blue, a basic dye, stains the acidic components with a focus on the cell nucleus. These dyes are combined with methyl alcohol, which dilutes the stock solution and buffers during the staining process. The blood components are stained in diverse shades between red and blue.

Applications: Leishman stain is frequently utilized when there is a requirement to analyze the Blood smear for multiple blood cell types, perform a Differential Leucocyte count, determine the type of Anemia, identify Toxic Granules, and assess the Platelet count, among other uses. It is also effective in distinguishing the nuclear and cytoplasmic morphology of different blood cells, such as Platelets, Red Blood Cells (RBCs), and White Blood Cells (WBCs), as well as detecting parasites. This stain is considered the most reliable choice for examining Peripheral blood films.

Preparing Leishman Stain in Laboratory

- Measure the weight of Leishman stain powder, around 0.15 g, using an analytical balance or weighing scale. Grind the powder in a Glass Mortar and transfer it into a brown bottle using a funnel.
- Pour approximately 20 ml of acetone-free methanol into the bottle through the same funnel, ensuring the dry stain is washed down.
- Recap the bottle and shake it in a circular motion for 2-3 minutes to help dissolve the stain crystals in the methanol solution. Warm the mixture at 50 °C for 15 minutes while shaking it occasionally.
- Add the remaining methanol to the mixture through the funnel, making the total volume 100 ml. Ensure every last drop of methanol is washed from the funnel into the stain mixture.
- Securely tighten the screw-cap on the bottle and continue shaking it for a few minutes.
- Keep the bottle closed without opening it or filtering its contents for approximately a week. After a week, it will be ready for use. Prior to use, filter the contents.
- Clearly label the bottle as Leishman Stock Solution, noting the batch number, the name of the person who prepared it, the date of preparation, and the expiry date. Document this information in the quality control logbook of your laboratory.
- Seal the bottle tightly to prevent the absorption of water vapors from the air and to prevent alcohol from evaporating. Store the bottle in a cool area away from direct sunlight.
- Do not store the solution near bottles containing acids. The Leishman Stain solution is stable for approximately three months when stored correctly.

Utilize the stock solution of Leishman stain in different dilutions, preferably in a ratio of 1 part stain to 2 parts buffer solution. The specific dilution should be determined based on the stain's quality and the staining's purpose. For instance, it can be used to examine blood cells in a thin smear or identify parasites in a thick smear.

Materials required:

a. Leishman Stain (Stock Solution)
b. Microscopic Glass Slide
c. Phosphate buffer (pH 6.8)
d. Graduated pipettes
e. Measuring cylinder
f. Distilled Water
g. Pasteur pipette
h. Coplin Jar
i. Blood Specimen – The blood specimen should be collected through a finger puncture (capillary puncture) or venipuncture using EDTA as an anticoagulant. The blood needs to be fresh and less than 1 hour old to achieve optimal results.

Staining Procedure:

- Begin by preparing a thin blood smear on a clean and dry microscope slide. Allow the smear to air dry.
- Once the smear is completely dry, cover it with an undiluted Leishman Stain solution. Count the drops of Leishman stain to ensure proper coverage.
- Allow the stain solution to stand on the smear for 2 minutes. This allows the methanol in the stain solution to fix the smear to the glass slide.
- After 2 minutes, add twice the amount of distilled water or Phosphate buffer solution to the smear. Mix the content by gently swirling or blowing. Incubate the slides for a minimum of 10 minutes at 37 °C. This step will stain the blood cells.
- Thoroughly rinse the slides with Phosphate buffer solution for up to 2 minutes or until they acquire a purple-pinkish tinge.
- Air dry the slides by tilting them to allow the water to drain off easily.
- Now, the smears can be mounted using a mounting medium such as Gurri's neutral mounting media or any other suitable medium that does not cause decolorization of the smear. Avoid using Canada balsam, as it may cause decolorization.
- Let the mounted smears dry in the air for a few hours, and then examine them under an oil immersion objective microscope lens.

Rapid Malaria Stain (JSB I & II)

Kit for Staining Malarial Parasite (Pack Insert)

PRINCIPLE

Polychromated Methylene Blue and Eosin stains specifically to basophilic and acidophilic cellular elements to demonstrate blood cells and hemoparasites.

STAINS COMPOSITION

1. RAPID-MALARIA JSB I	**500 mL**
Methylene blue	4.3 mMol/L
Sulphuric Acid	1%
Potassium Dichromate	0.5 gms
Phosphate Buffer	0.1 mMol/L
Contains polychromating materials, preservatives and stabilizers.	
2. RAPID-MALARIA JSB II	**500 mL**
Eosin	4.3 mMol/L
Phosphate	0.1 mMol/L
Contains preservatives and stabilizers.	
3. EASYFIX	**50 mL**
Peripheral Smear Fixative	

Preparation of Working Reagent

All Reagents are ready to use.

STORAGE AND STABILITY

All reagents are stable between 25 to 35°C for 60 months. RAPID-MALARIA FIXATIVE must be stored tight capped away from heat and fire. Rapid-MALARIA JSB STAIN may be exhausted on prolonged use over 150 smears. It is advised to discard Rapid-MALARIA JSB Stain as and when the smear gets lighter stain.

SAMPLE

Peripheral blood samples on clean glass slides may be collected upon febrile episodes from susceptible patients. K_3 EDTA may be used without substantial change in staining results.

THIN SMEAR

A drop of blood can be spread with a fine edge spreader. Dry the smear at air and fix it with FIXATIVE or rectified spirit within 4 hours. Fixed smear can be stored for longer period without much variation in staining result, however, it is preferable to stain all smears as early as possible.

THICK SMEARS

In mass or conclusive screening it is preferable to prepare thick smears by collecting 1-2 drop of blood on a clean glass slide and spread it to the shape of a coin. Dry at air, add few drops of distilled water to the slide to cover the smears and wait for 1-2 minutes to dehaemoglobinize the smear. Carefully remove water and dry at air. Fix the smear

LIMITATIONS

The performance of stain must be periodically checked by known positive and known negative samples. The accuracy of reporting Malarial parasite is subject to the professional experience of each person as well as the use of a good optical system that could make clear magnification of the parasite from the smear.

PROCEDURE

Fill up two Coplin jars or wide mouth bottles with **Rapid-MALARIA STAIN - I and II**

A . FOR THIN SMEARS

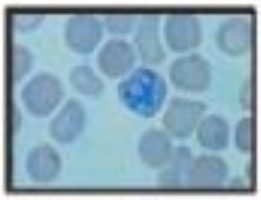
1. Spray RAPID-MALARIA JSB STAIN FIXATIVE to thin smears and dry at air.
2. Dip fixed smear to **RAPID-MALARIA JSB STAIN - II** for 3-5 seconds and wash in running tap water.
3. Dip smear in **RAPID-MALARIA JSB STAIN - I** for 35-45 seconds. Wash in running tap water. Dry at air and see under oil immersion objective for malarial parasites. Staining time in each stain may be increased or decreased according to individual staining result.

B. FOR THICK SMEARS DEHAEMOGLOBINIZING

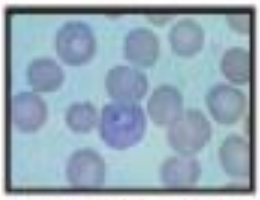
1. Arrange air dried unfixed thick smears in a slide rack keeping the smear side up.
2. Add few drops of distilled water to the slide to cover the smears and wait for 1-2 minutes to dehaemoglobinize the smear. Carefully remove water and dry at air and fix smear by spraying fixative. Dry at air.
3. Dip fixed smear to RAPID-MALARIA JSB STAIN - II for 5-6 seconds and wash in running tap water.
4. Dip smear in RAPID-MALARIA JSB STAIN - I for 35-45 seconds. Wash in running tap water. Dry at air and see under oil immersion objective for malarial parasites.

RESULTS

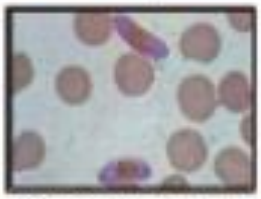
Leucocyte nuclei	-	varying shades of dark blue
Leucocyte Cytoplasm	-	pale or light blue
RBC	-	pale reddish
Malaria Parasite	-	Varying shades of blue
Schiffner's Dot	-	dark bluish red.
Platelets	-	pale or dark blue.



Pl. vivax trophozoites



Pl. Vivax trophozoites



Pl. Falciparum Gametocyte

Pl. falciparum trophozoite

BULK STAINING PROCEDURE

Using staining tanks number of slides can be stained with relatively less time.

A. THIN SMEAR

1. Label all smears with appropriate patient identification marks.
2. Fix thin smear by immersing or spraying with Rapid-MALARIA JSB STAIN Fixative and arrange in a slide holder.
3. Fill Rapid-MALARIA JSB STAIN II in a suitable staining jar and dip the smears in the stain for 3-5 seconds.
4. Remove and dip in another jar filled with water for 10-20 seconds. Agitate the slide holder to remove excessive stain from the smear.
5. Remove the water by keeping the slides holder over a filter paper.
6. Fill Rapid-MALARIA JSB STAIN - I in a suitable staining jar and dip the smears in the stain for 35-45 seconds.
7. Remove and dip in another jar filled with water for a minute. Agitate the slide holder to remove excessive stain from the smear. Dry the smears at room temperature or in an oven thermostatically controlled to 50-60°C.

B. THICK SMEAR

1. Label all smears with appropriate patient identification marks and arrange in a slide holder.
2. Dehaemoglobinize by dipping in a jar containing distilled water for 1-2 minutes. Carefully remove from water.
3. Proceed to Bulk staining procedure thin smear step No. 2.

NOTE : Unfixed thick smears may run out from the slides therefore, all steps of Dehaemoglobinization and washing must be without much agitation.

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J-245, MIDC, TARAPUR, BOISAR - 401 501, MAHARASHTRA

E-mail : biolab@vsnl.com / www.biolabdiagnostics.com

Customer Care : (+ 9122) 28088243

Giemsa staining

This procedure was developed to detect blood parasites like Trypanosoma, Filaria Malaria etc.; this procedure is now used to determine blood cell counts. Initially designed for efficient detection of malarial parasites in blood smears, it is now used for other blood parasites like trypanosomes, Microfilaria, etc., and for determining blood cell counts. It is commonly recommended for detection and identification of blood parasites.

Preparation of Giemsa Stain Reagents manually:

1. **Stock 100× Giemsa Buffer** – 0.67 M
    a. Na2HPO4: 59.24 g
    b. NaH2PO4H2O: 36.38 g
    c. Deionized water: Up to 1000.00 ml

The options for achieving sterility are autoclaving or filter-sterilizing with a 0.2 µm pore size filter. The sterile buffer can be stored at room temperature for one year without losing its stability.

2. **Working Giemsa Buffer** – 0.0067M, pH 7.2
    a. Stock Giemsa Buffer: 10.0 ml
    b. Deionized water: 990.0 ml

Check pH before use. It should be 7.2. Stable at room temperature for one month.

3. **Triton X-100 5%**
    a. Deionized water (warmed to 56°C): 95.0 ml
    b. Triton X-100: 5.0 ml

    Prewarm the deionized water and slowly add the Triton X-100, swirling to mix.

4. **Stock Giemsa stain** (Giemsa stain is available commercially, but the following formulation gives more constant results and does not expire)
    a. Glass beads, 3.0 mm: 30.0 ml
    b. Absolute methanol, acetone-free: 270.0 ml
    c. Giemsa stain powder (certified): 3.0 g
    d. Glycerol: 140.0 ml

Prepare Stock Giemsa Stain solution as follows:

- To prepare the Giemsa stain, first, place the glass beads and remaining ingredients in the order specified into a 500 ml brown bottle. It is important to use clean and dry glassware during this process. Securely tighten the cap of the bottle.
- Next, place the bottles at an angle on a shaker and gently shake them for 30 to 60 minutes each day for at least 14 days.

- The stock Giemsa stain can be stored indefinitely at room temperature, provided it is tightly stoppered and moisture-free. Stock stain quality may even improve with age.
- Before using the stain, make sure to shake the bottle thoroughly. Filter a small amount of the stock stain through a Whatman number 1 filter paper into a test tube. From this tube, use a pipette to prepare the working Giemsa stain.

Working Giemsa stain (2.5%): Prepare fresh for each batch of smears

a. Working Giemsa buffer: 39 ml
b. Giemsa Stain Stock: 1 ml
c. 5% Triton X-100: 2 drops

Staining Procedure:

- Follow the instructions given above to prepare a fresh working Giemsa stain in a staining jar. For a Coplin jar, 40 ml of stain is sufficient. Adjust the volume for other jar sizes, but ensure that proportions are not changed.
- In a separate staining jar, pour 40 ml of working Giemsa buffer and add two drops of Triton X-100. Adjust the volume based on the size of the jar.
- Submerge the slides into the working Giemsa stain (2.5%) for a duration of 45-60 minutes.
- Remove thin smear slides and rinse them by dipping them 3-4 times in the Giemsa buffer. Thick smears should be left in the buffer for 5 minutes.
- Allow the slides to dry upright on a rack.

Note: Instead of staining the smears for 45-60 minutes in 2.5% Giemsa stain, more concentrated stains could be used for shorter periods to produce faster results. For instance, an alternative would be to use 10% Giemsa stain for 10 minutes. However, shorter staining times require more stains and may result in less predictable, less-quality staining.

Quality Control of staining procedure:

To ensure the accuracy of staining results, it is recommended to include a positive smear (such as one for malaria) when preparing a new batch of working Giemsa stain. Since commercially available quality control smears are not readily accessible, it is possible to prepare them from a patient's blood and store them for future use. The following method can be employed for preparing and storing these smears:

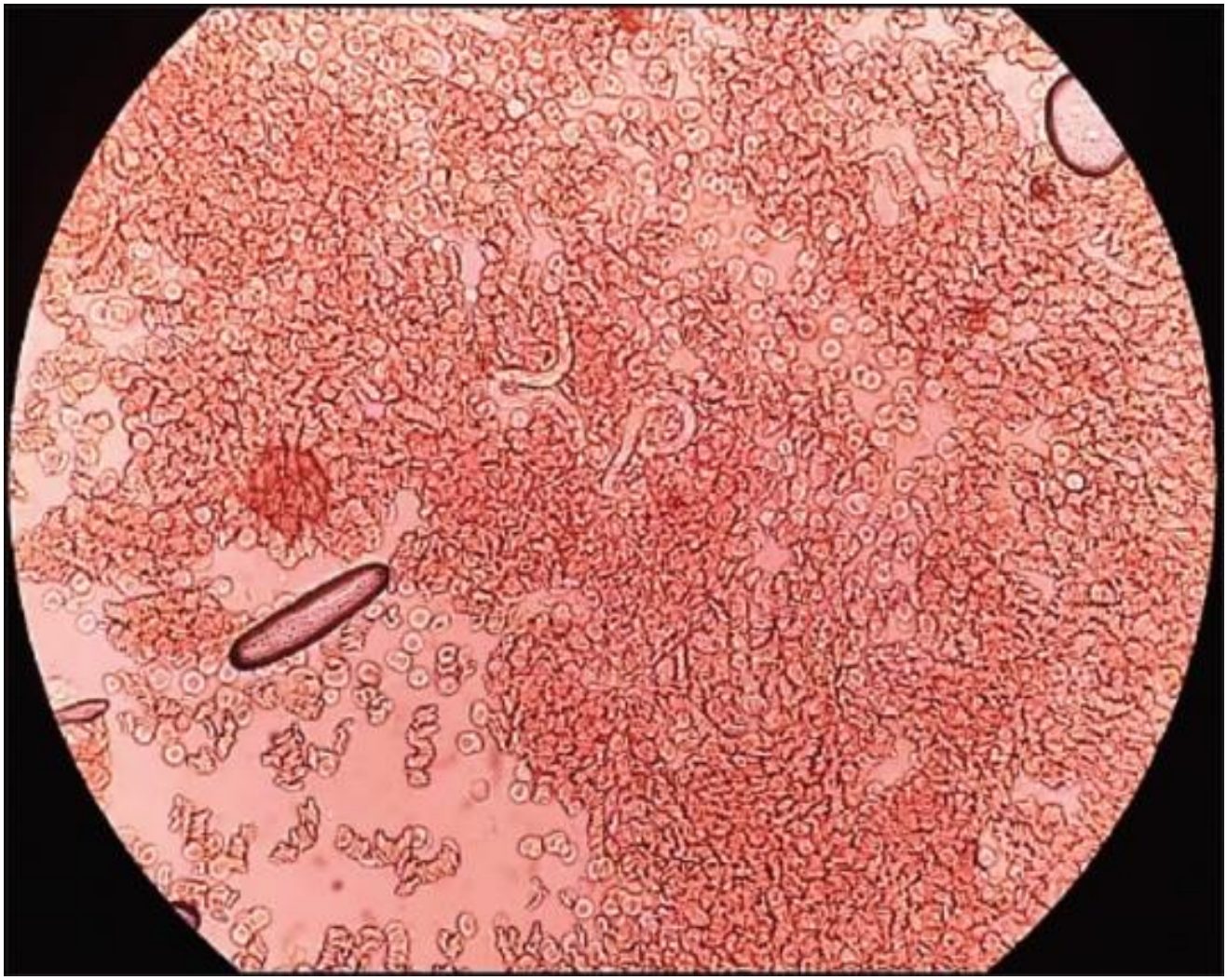
- Select a blood specimen from a patient that has been anticoagulated with EDTA. Ensure the specimen contains enough parasites, with at least one parasite visible in every 2 to 3 fields.
- Prepare as many thin smears as possible within one hour of drawing the blood from the patient.

- Speed up the drying process of the smears by using a fan or blower at room temperature.
- Fix the smears by allowing them to dry after applying absolute (100%) methanol.
- Place the smears in a box, touching front to back, without separating grooves.
- Label the outside of the box with the species, date, and "Giemsa control slides."
- If the purpose is to create quality control slides, store the box at a temperature of -70°C or colder.
- Before use, remove a smear from the box and allow any condensation to evaporate. Label the slide as "+ malaria" along with the current date. The smear is now ready for staining, as it has been previously fixed.

Examining Thick Smears

- In order to screen for parasites and detect mixed infections, the thick smear is useful since the erythrocytes (RBCs) have been lysed, and the parasites are more concentrated.
- Screening the entire smear at a low magnification (10× or 20× objective lens) is done to identify larger parasites like microfilaria.
- Switch to the 100× oil immersion objective lens to examine the smear. Look for an area that is well-stained, without any stain precipitate, and has a good number of white blood cells (WBCs) present (around 10-20 WBCs per field).
- If parasites are visible, determine the species based on the thick smear and then examine the thin smear for species identification. Usually, the thin smear is the preferred sample for determining the species.
- For malaria diagnosis, the World Health Organization recommends that at least 100 fields, each containing approximately 20 WBCs, should be screened before considering the thick smear negative ("No Parasites Found" or NPF). This threshold ensures a sensitivity of detecting at least four parasites per microliter of blood, assuming an average WBC count of 8,000 per microliter. However, in nonimmune patients, symptomatic malaria can occur at lower parasite densities, so screening a larger number of fields (e.g., 200, 300, or the entire smear) may be necessary depending on the clinical context, availability of laboratory personnel and time constraints. National Committee for Clinical Laboratory Standards recommends examining at least 300 fields using the 100× oil immersion objective.
- Microfilariae, which are night wanderers, usually circulate between 10 PM and 2 AM - corresponding to the usual feeding time of mosquitoes. The prevalence rate of microfilariae is approximately 30% in children under ten years and nearly 69% in adolescents under 19 years. Two thin and two thick smears are prepared and stained with Giemsa or Haematoxylin to identify microfilariae. Species

determination can be done based on specific morphological characteristics. Scanning the entire blood film at ×10 magnification before reporting it as negative is recommended.



Examining thin Smears

Thin smears serve multiple purposes, including identifying the species of parasites that have been found on thick smears, screening for parasites in situations where there aren't enough thick smears available, and providing a quick initial screen while the thick smear is drying. If the low magnification examination (using a 10× or 20× objective lens) has not been performed on thick smears, it should be done on the thin smears. Next, carefully analyze the smear using the 100× oil immersion objective lens. According to the standards set by the NCCLS, examining a minimum of 300 fields using the 100× oil immersion objective lens ensures thorough evaluation.

Quantifying parasites

- In certain cases, especially for malaria, quantifying the number of parasites can provide valuable clinical information. If the physician requires this information, malaria parasites can be quantified relative to blood components such as red blood cells (RBCs) or white blood cells (WBCs).
- To quantify malaria parasites against RBCs, count the infected RBCs among a range of 500-2,000 RBCs on the thin smear. Express the results as the percentage of parasitemia using the following formula: % parasitemia = (number of parasitized RBCs / total number of RBCs) × 100
- For high parasitemia (e.g., >10%), examine 500 RBCs. For low parasitemia (e.g., <1%), examine 2,000 RBCs or more. It's important to count asexual blood stage parasites and gametocytes separately, as only the former are clinically significant. Note that gametocytes of P. falciparum can persist even after asexual stages have been eliminated by drug treatment.
- To quantify malaria parasites against WBCs, tally the parasites against WBCs on the thick smear. You need to count 1,000 WBCs or 500 parasites. Record the results as parasites per microliter of blood, using the WBC count if known. In the absence of a known WBC count, assume an average of 8,000 WBCs per microliter of blood.
- Parasites per microliter of blood are equal to the number of parasites/number of WBCs × WBC count per microliter (or assume 8,000 if the WBC count is unknown)
- The results of % parasitized RBCs and parasites per microliter of blood can be converted between each other if both the WBC count and RBC count are known. If these counts are not available, ideally, you can assume 8,000 WBCs and 4,000,000 RBCs per microliter of blood.

Detection of Malarial Parasite in a stained blood smear

Leishman staining *is useful to detect Malarial parasites. Thick and thin smears are also used in many laboratories. The methods of making thick and thin smears have already been mentioned above. After staining, the smear is examined under the microscope to detect and identify the malarial parasite and its species.

In India, mainly two species are prevalent

- Plasmodium vivax
- Plasmodium Falciparum

Plasmodium falciparum

It is widespread in the tropics and often represents high parasitemia. In blood films, only trophozoites and gametocytes are usually seen.

Trophozoites

These are small rings, occasionally larger rings in heavy infections. Parasites are difficult to recognize if the patient has taken antimalarials. Trophozoites of resistant strains can appear thick and distorted. They are often with double chromatin dots. The host cell may have more than one trophozoite.

Schizonts

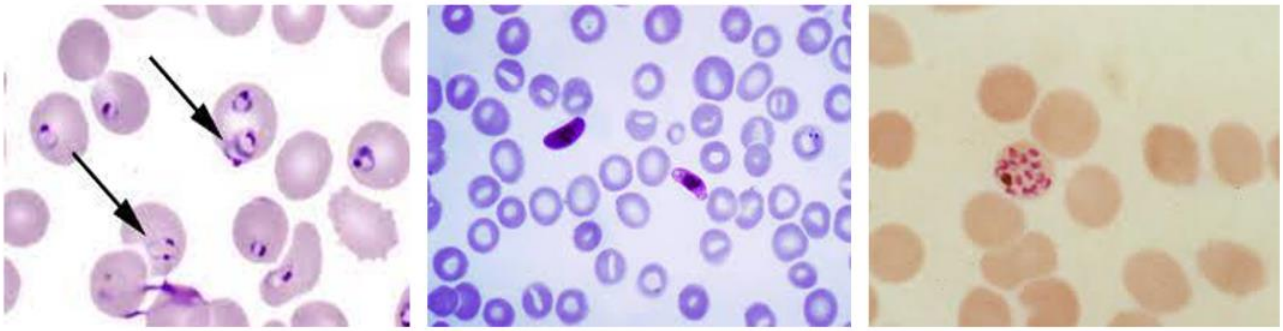
They can be seen occasionally in severe infections

- Usually with 2 or 4 merozoites and pigment.

Gametocytes

Gametocytes are usually banana-shaped. However, rounded forms may be seen if the film dries slowly.

Plasmodium falciparum



Trophozoites Gametocyte Schizoint

Plasmodium vivax: P. Vivax is widespread in the tropics and temperate areas. Rarely more than 2% of cells are infected. In blood smears, Trophozoites, schizonts, and gametocytes areusually seen.

Trophozoites: They are large and amoeboid with fine pigment. Cytoplasm fragmented in thick films.

Schizonts: They are Large, round, or irregular in form. It may contain up to 24 or more merozoites and fine pigment.

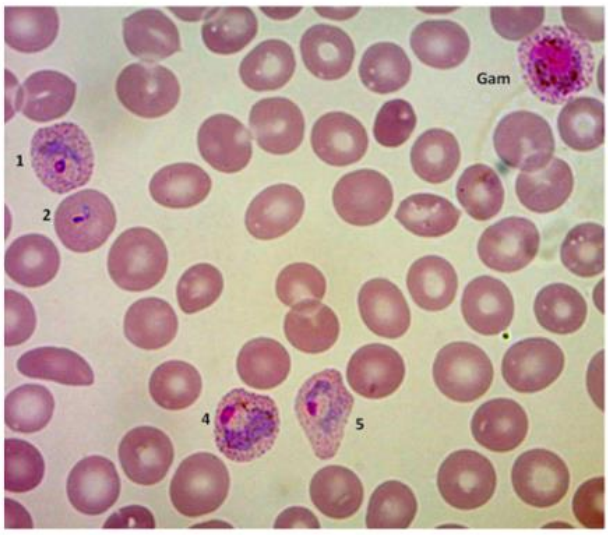
Gametocytes

- Gametocytes are large, round, or irregular in form (small forms also) with scattered pigment.

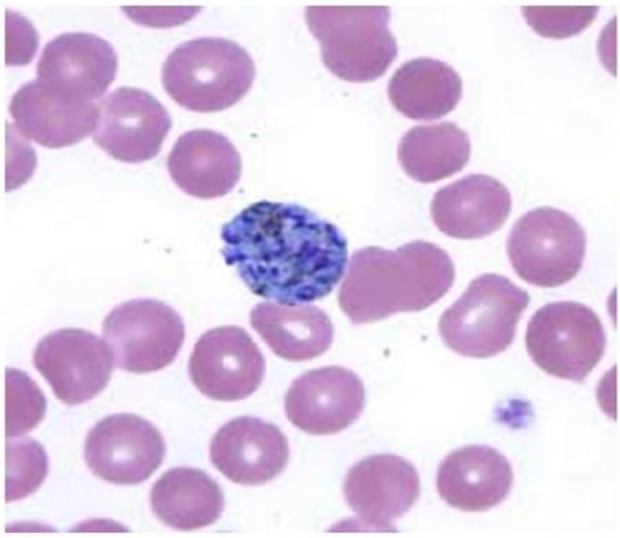
Host cell

- In the case of P. vivax, the host cell is enlarged, irregular in shape, and has pale staining.

Plasmodium vivax

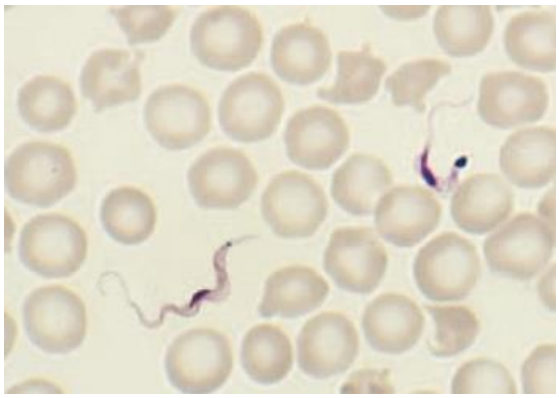


Trophozoites (1-5) and Gametocyte



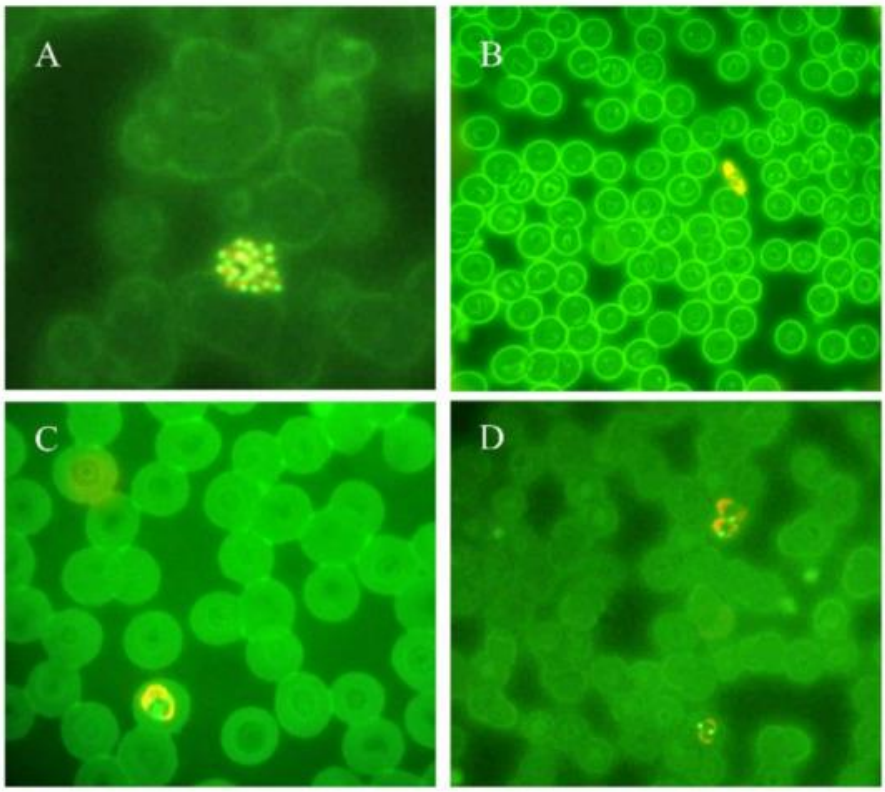
Schizoint

Detection of Trypanosomes in Blood Smear:



Detection of blood parasites using fluorescent dyes

Fluorescent dyes have been utilized to identify blood parasites by staining nucleic acids. The Kawamoto technique involves staining blood smears on a slide with acridine orange. The slide is then analyzed using either a fluorescence microscope or a light microscope that has been equipped with an interference filter system. This staining procedure differentially stains nuclear DNA in green and cytoplasmic RNA in red, enabling the recognition of blood parasites. This method has been successfully used to detect malarial parasites and, to a lesser extent, African trypanosomes.



Thin blood smears of blood samples from malaria cases prepared and stained with acridine orange. (**A**) *P. vivax* schizont (100×). (**B**) *P. falciparum* gametocyte (40×). (**C**) *P. ovale* trophozoite (100×). (**D**) *P. vivax* trophozoites (100×). (Picture by A. Calderaro, Department of Medicine and Surgery, University of Parma, Parma, Italy).

Ziehl Neelsen Acid Fast staining:

Purpose: Direct acid-fast bacilli (AFB) detection in sputum sample smear using the Ziehl–Neelsen staining procedure.

Introduction

This procedure stains the *Mycobacterium tuberculosis* complex and other non-tubercular *mycobacteria* (NTM). Acid-fast staining is a differential staining technique that differentiates between Acid-fast and Non-acid fast microbes. Some organisms like Mycobacterium are called Acid fast because they have **mycolic acid** in their cell walls. They are not stained easily and need some harsh methods involving a heat process like in the Ziel-Neelson staining method. But once they are stained, they resist decolorization even with strong acids, i.e., 20% sulphuric acid, and retain the primary dye, Carbol fuchsin. That is why they are known as Acid-fast organisms. It is assumed that mycolic acid prevents acid from decolorizing protoplasm. The microbes which are decolorized by acid or acid-alcohol decolourizers are known as Non-Acid-Fast.

Detection limit: The smear detection limit is 10^5 bacilli /ml of samples.

Primary Sample: Sputum: spontaneous/induced: An early morning specimen generated after a bout of cough.

Type of Container: Collect in a sterile leakproof, wide-mouth container.

Collection of Sputum-spontaneous/induced: An early morning specimen generated after a bout of cough is to be collected. The patient is instructed to brush his or her teeth or remove artificial dentures if any, and gargle with water immediately before obtaining the sputum specimen to reduce the number of contaminating oropharyngeal bacteria.

A sterile screw-cap container with a capacity of approximately 100 ml should be used to obtain a specimen resulting from a deep cough. The patient should be instructed to press the rim of the container under their lower lip to ensure that the entire cough sample is collected, preventing contamination of the outside of the container. After the cough sample has been collected, the cap of the container should be tightly screwed on.

Suppose any material spills onto the outside of the container. In that case, it should be carefully wiped off using a tissue that has been moistened with a disinfectant. However, ensuring no disinfectant enters the container is important to avoid interference with the specimen.

Early-morning sputum samples are preferred for optimal results, as they contain pooled overnight secretions, which are more likely to have a higher concentration of pathogenic bacteria. It is discouraged to collect sputum samples over 24 hours.

An induced sputum sample is obtained by inhaling warm, aerosolized hypertonic (5-10%) saline, which irritates the lungs enough to induce coughing and produce a thin, watery specimen.

Sample transportation: The specimen is to be delivered to the laboratory as early as possible, preferably within 2 hours. In case of delay, the sample should be kept in the refrigerator till it is submitted to the laboratory. The transportation should be done in a leakproof insulated box.

Sample Rejection Criteria: Under situations the sample is rejected

- Samples that are watery or contain only saliva.
- Unlabelled sample.
- Soiled container or request form.

The rejected sample data is recorded as per prevailing rules

Safety Precautions: Process all specimens in the Biosafety cabinet IIA using standard precautions.

Ziehl Neelsen stain Preparation

Carbol Fuchsin (0.3%)

Dissolve 7.5 gm of Basic Fuchsin in 250 ml of 95% ethanol.

Dissolve 112 ml of molten phenol crystals in 500 ml of distilled water, heating gently.

Mix the above solutions and makeup to 2500 ml with distilled water. Filter using Whatman Filter No 1.

Dispense in stain dispensing bottles.

Acid alcohol (3%)

Carefully add 3 ml of concentrated HCl to 97 % ethanol; mix gently. Dispense in stain dispensing bottles.

Methylene blue (0.3%)

Dissolve 0.3 g of methylene blue chloride in 100 ml of distilled water. Filter using Whatman Filter No 1.

Dispense in stain dispensing bottles.

Quality control of the staining method is performed daily using known acid-fast positive (*M.tuberculosis*) and known acid-fast negative sputum samples, and the results are recorded

Reagents:

- ZiehlNeelsen stain solutions (prepared as above) Water dispensing bottle
- Discard jar containing disinfectant/ Paraffin oil/ cedarwood oil

Equipment and other materials

- Biosafety cabinet class II A
- Gas Burner
- Glass Marking Pencil (diamond Pencil), Wooden Sticks, or inoculation loop
- Glass Slide rack
- Match Box/Gas Lighter Glass Slide
- Glass rods are fixed on a wash basin to hold the slides during staining.
- PPE like N95 mask, Gloves, Laboratory Coat etc.
- Good Binocular Microscope

Procedure: Smear preparation

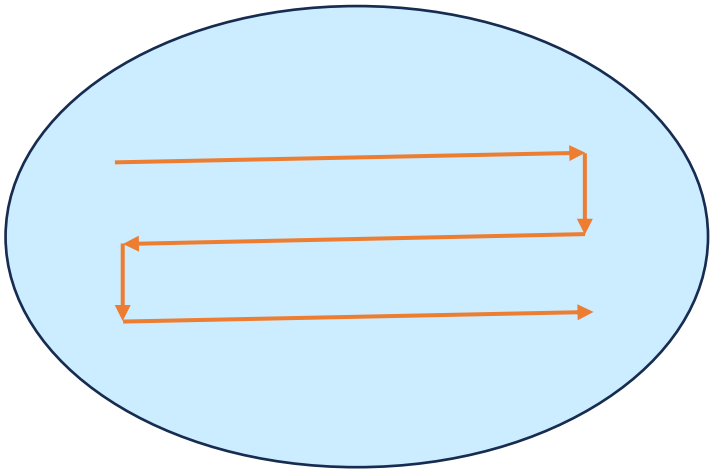
- The smears should be prepared in the Biosafety cabinet.
- New, clean, and unscratched microscopic slides are used.
- Label one end of the slide with the Patient ID or laboratory serial number.
- Use a disposable inoculation loop or wooden stick to prepare the smear.
- After use, the disposable inoculation loop or sticks are discarded in a disinfectant solution.
- Smears are air-dried and then heat- fix by passing slide over the flame three times, smear side up.
- Don’t stack the slides with a smear.

Ziehl- Neelsen Staining Method

- Place a heat-fixed slide with a smear on the glass rods fixed on the wash basin.
- Flood the slide with hot 0.3% carbolfuchsin
- Either heat the slide from the lower side using a spirit lamp until the stain on the slide starts steaming. Avoid boiling. Or beforehand, heat the stain solution at about 85°C and pour on the slide to cover the smear completely.
- Wait for 8 minutes and wash briefly with water.
- Decolorize by flooding with 3% acid alcohol for 4 minutes. Wash smear again
- Pour 0.3% methylene blue to counterstain for 2 minutes. Rinse with water,
- Dry in air
- Examine under a microscope using oil immersion (100x)

Reporting and Interpretation

Examine the slide under the oil immersion lens thoroughly for the presence of AFB, which will appear pink coloured, approx. 0.3 to 3µm long, slender, beaded, or barred slender rods against the blue background. The recommended procedure for smear examination is as follows. Lines with arrows indicate the movement pathway of the field observation by moving the slide under the microscope's lens.



Sputum smears are examined and interpreted as indicated in the table below:

Examination finding	Result as recorded	Grading	No. of fields examined
> 10 AFB per oil immersion field	Positive	3+	20
1–10 AFB per oil immersion field	Positive	2+	50
10–99 AFB per 100 oil immersion fields	Positive	1+	100
1–9 AFB per 100 oil immersion fields	Positive	Scanty*	100
No AFB in 100 oil immersion fields	Negative	Negative	100

** Record exact number seen in 100 fields*

Limitations:

ZN staining procedure gives positive result only if the sputum sample contains more than 10^5AFB/ml. The samples with the number of AFB less than 10^5AFB/ml may show negative results. Hence if the smear is reported to be negative once, it may not be considered that the patient is negative for AFB. In such cases, more samples need to be tested.

False positive results:

False positive results may be seen under the following circumstances:

- Use of scratched slides for smear preparation.
- Improperly cleaned slides.
- Improperly cleaned containers.
- Allowing the stain to dry and crystallize on the slide. Under decolorization of smears.
- Transfer of acid-fast bacilli via oil immersion objective, if not wiped with a clean tissue paper after examining positive smear.
- Tap water may be a source of environmental *mycobacteria*—unfiltered or boiled stain.
- Reporting by an unskilled person.

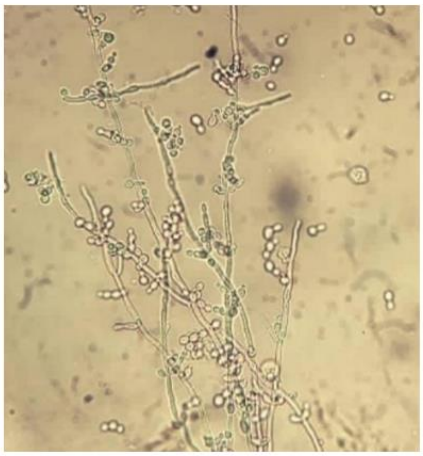
False-Negative Results

False Negative results may be seen under the following circumstances:

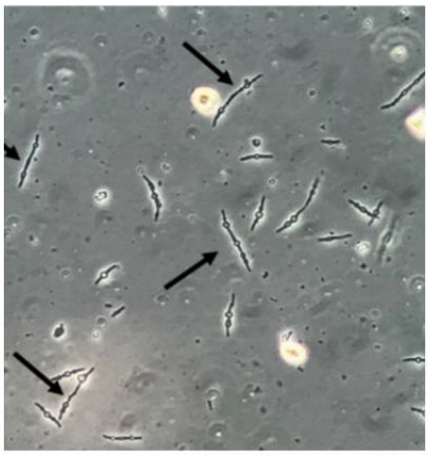
- Inadequate sample.
- Improper examination of smears.
- Improper staining.
- Reporting by an unskilled person.

Urine Microscopy for Detection of Microbes:

Fungi like Cryptococcus or Candida and bacteria like Haemophilus and Pneumococcus can be detected in urine samples very easily, as shown below:



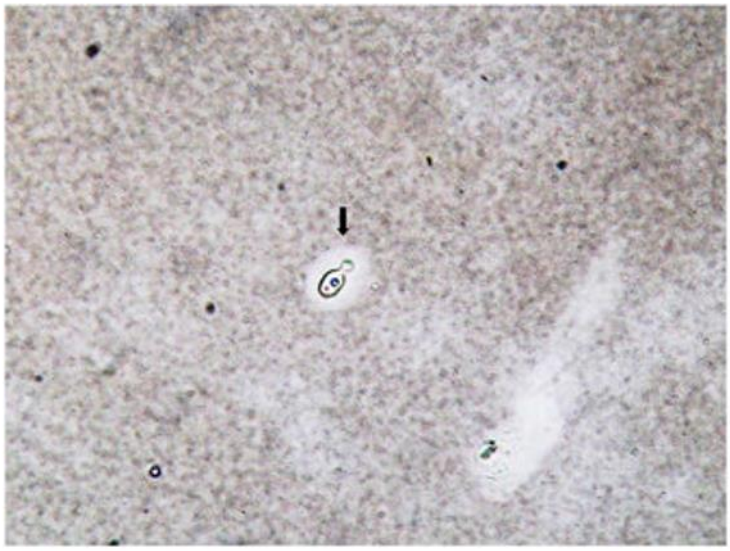
Candida albicans in Urine sample



MDR Esch. coli forming filamentous forms and spheroplasts in urine samples Phase contrast Microscopy)

Cryptococcus Species In Urine

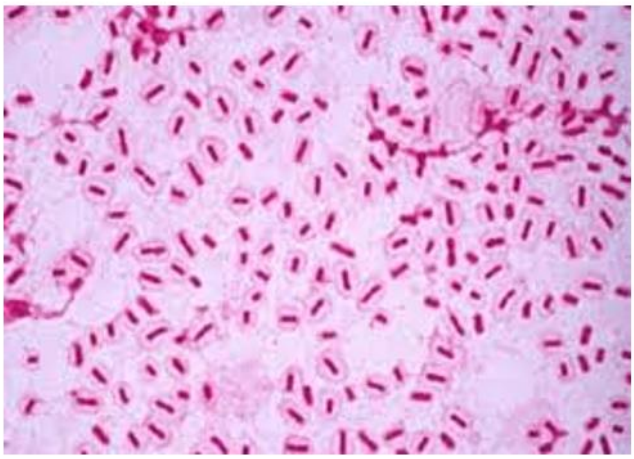
Non-*neoformans* cryptococci, such as *Cryptococcus laurentii* and *Cryptococcus albidus*, are emerging as opportunistic pathogens, causing disease in patients with impaired cell-mediated immunity (eg, HIV-infected patients or those with hematologic malignancies), in those using steroids or chemotherapeutic agents, and in those with invasive devices. Photograph of a microscopic image of Cryptococcus species in urine sample is as below;



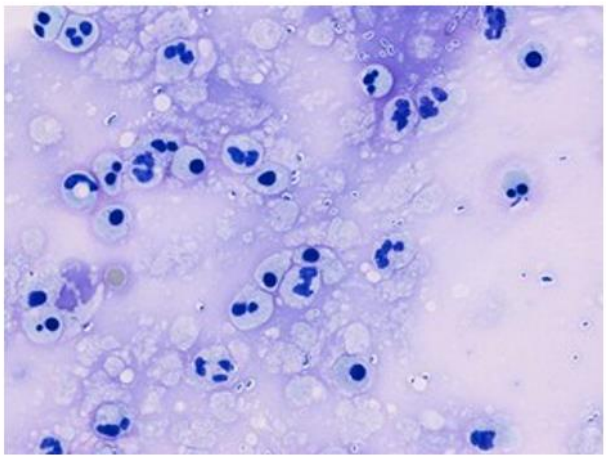
Cryptococcus sp. encapsulated yeast (arrow) on the urine sediment stained with China ink. Bright-field microscopy.

Microbial Detection by Microscopy In CSF

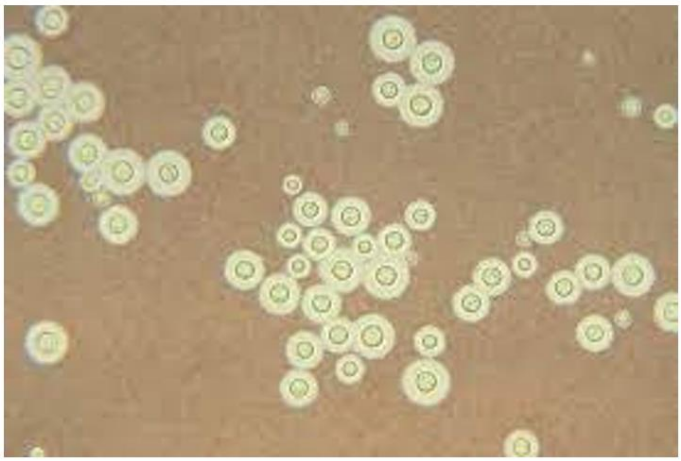
Fungi like Cryptococcus or Candida and bacteria like Haemophilus and Pneumococcus can be detected in CSF samples very easily, as shown below:



Haemophilus influenzae in CSF

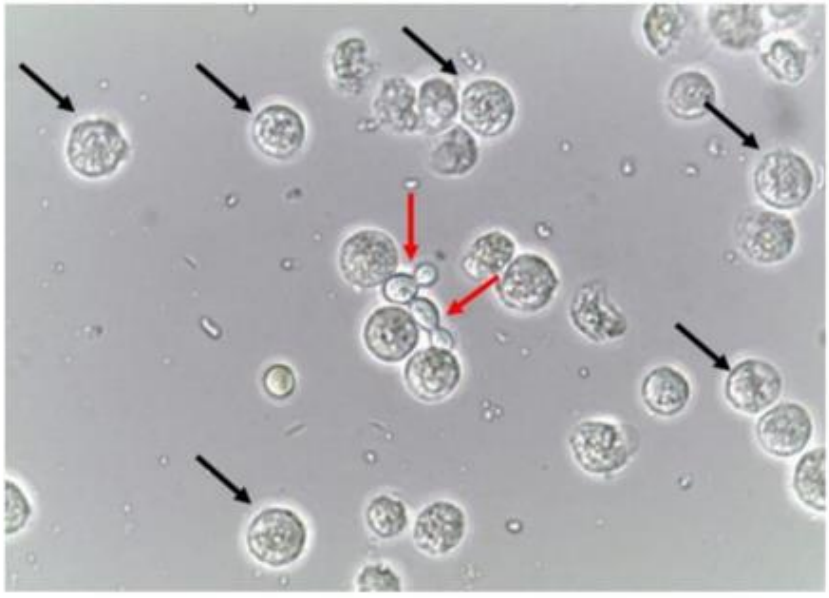


Pneumococcus pneumoniae in CSF



Cryptococcus in CSF (India ink preparation)

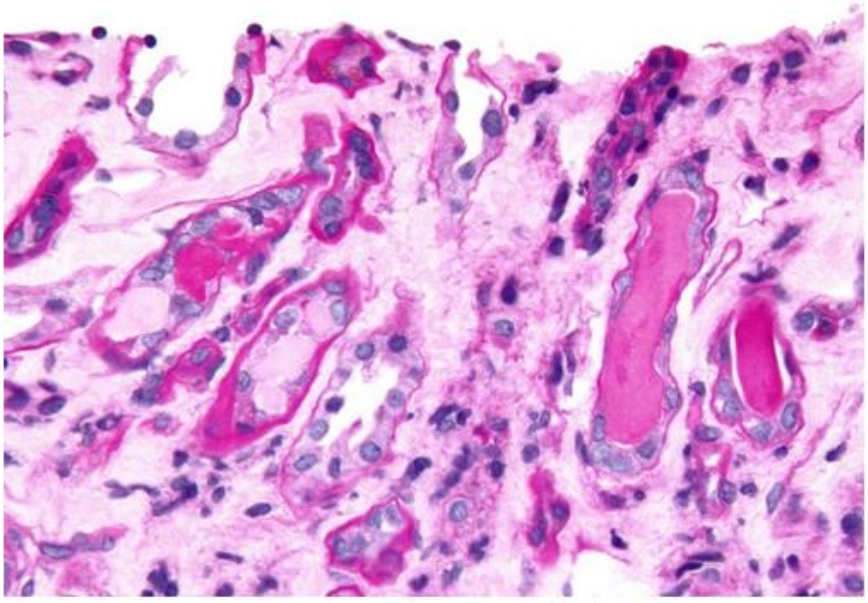
Pus Cells and Yeast Cells in Urine Sample (Seen Under A Microscope)



Pus Cells (Black Arrow) and Yeast Cells (Red Arrow) in Urine Sample (Seen Under A Microscope)

Urinary Casts:

Urinary casts are tiny tube-shaped structures that may be found when urine is examined under the microscope during a Microscopic urinalysis test. Kidney cells, White blood cells, red blood cells, or substances such as protein or fat contribute to the formation of Urinary Casts. The below-given table depicts different types of casts in urine:



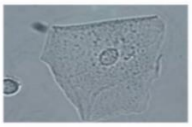
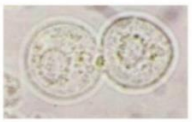
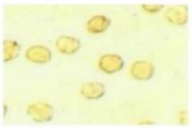
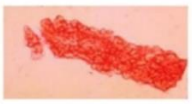
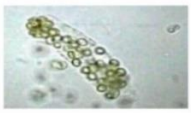
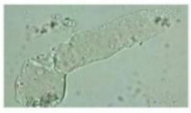
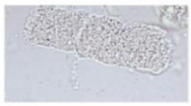
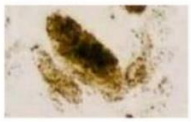
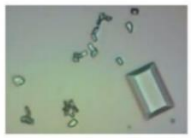
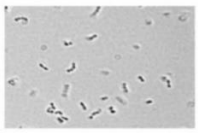
Origin or urinary casts in kidney

Type of Cast	Condition(s) Associated
Hyaline casts	Normal urine; Dehydration; Fever; Exercise
Cellular casts	Glomerulonephritis; Pyelonephritis; Interstitial nephritis; Acute tubular necrosis
Granular casts	Acute tubular necrosis
Waxy casts	Advanced kidney disease
Fatty casts	Nephrotic syndrome
Broad casts	Chronic renal failure; Nephrotic syndrome
Epithelial casts	Acute tubular necrosis; Toxic nephropathy
Mixed casts	Various kidney diseases
Red blood cell casts	Glomerulonephritis; Vasculitis
White blood cell casts	Pyelonephritis; Acute interstitial nephritis

Interpretation of Urine Casts:

Dr. Gerald Diaz has elaborated on the interpretation of urinary castes, which is as below;

Table 4 Interpretation of urine microscopy findings

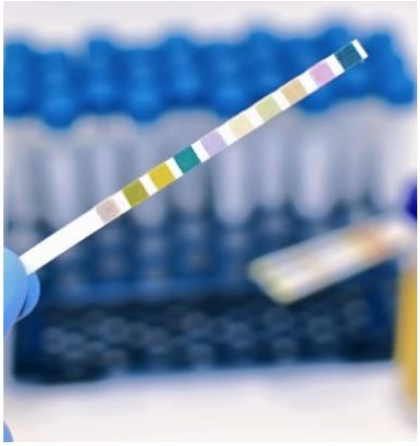
Microscopy finding	Example	Significance
Epithelial cells		Normal
Renal tubular cells		Acute tubular injury
Non-dysmorphic red cells		Non-glomerular bleeding from anywhere in the urinary tract
Dysmorphic red cells		Glomerular disease, but can also be seen if urine sample is not fresh at time of microscopy
Red cell casts		Diagnostic of glomerular disease
Leukocytes		Up to 3 per high-power field = normal; >3 per high-power field = inflammation in urinary tract
White cell casts		Renal infection
Hyaline casts		Any type of renal disease
Granular casts		More significant renal disease
"Muddy brown cast"		Necrotic tubular cells aggregated with tamm horsfall protein indicating acute tubular injury
Crystals		Some crystals can be found in healthy individuals; "abnormal" crystals may indicate metabolic disorders or excreted medications
Bacteria		Urinary tract infection; contamination

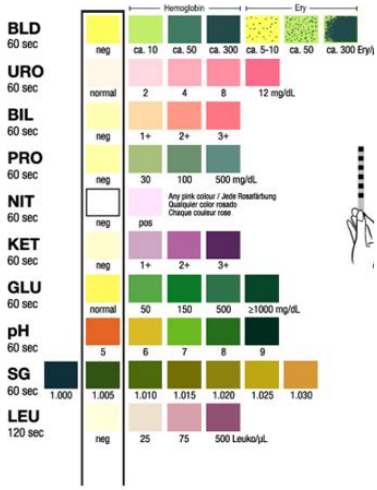
Urine Analysis By Dipstick

A dipstick is a thin, plastic flat strip with strips of reagents on it. On placing in the urine sample, the regent strips/lines change the colour, and the colour intensity is directly proportional to the concentration of test parameters, particularly when their levels are above typical levels. A dipstick urine test is normally done to check for:

- pH
- Specific gravity
- Sugar
- Protein
- Evidence of infection
- Ketones
- Bilirubin

An example of a urine dipsticksis given below:





Urine Dipstick Testing: Causes of False-Positive and False-Negative Results

Dipstick test	False-positive test	False-negative test
Bilirubin	Phenazopyridine (Pyridium)	Chlorpromazine (Thorazine), selenium
Blood[1]	Dehydration, exercise, hemoglobinuria, menstrual blood, myoglobinuria, semen in urine, highly alkaline urine, oxidizing agents uses to clean perineum	Captopril (Capoten), elevated specific gravity, pH <5.1, proteinuria, vitamin C, dipstick exposed to air
Glucose	Ketones, levodopa (Larodopa), dipstick exposed to air	Elevated specific gravity, uric acid, vitamin C
Ketones	Acidic urine, elevated specific gravity, some drug metabolites (e.g., levodopa)	Delay in examination of urine
Leukocyte esterase[3]	Contamination,[2] nephrolithiasis	Elevated specific gravity, glycosuria, ketonuria, proteinuria, cephalexin (Keflex), nitrofurantoin (Furadantin), tetracycline, gentamicin, vitamin C
Nitrites	Contamination, exposure of dipstick to air	Elevated specific gravity, elevated urobilinogen levels, nitrate reductase-negative bacteria, pH <6.0, vitamin C
Protein[4]	Alkaline or concentrated urine, quaternary ammonia compounds, iodinated radiocontrast agents	Acidic or dilute urine, primary protein is not albumin, such as Bence-Jones protein
Specific[5] gravity	Dextran solutions, IV radiopaque dyes, proteinuria	Alkaline urine
Urobilinogen	Elevated nitrate levels, phenazopyridine	

1. Test depends on peroxidase activity of RBC. Tests will be positive with intact or lysed cells. This test is very sensitive and may be positive in normal urine (1-2 RBC/HPF).
2. Especially vaginal contamination.
3. Sterile pyuria seen with interstitual nephritis, TB, and nephrolithiasis.
4. Not clinically significant unless 3 + or greater. Detects mainly albumin and requires protein excretions of 300-500 mg/day.
5. Accurate analysis for osmolality requires osmometer.

Adapted from *Am Fam Physician* 2006;74(7):1096.

The urine dipstick is dipped/soaked in the urine sample, and the results are recorded immediately within few minutes or as per the manufacturer instructions One may refer to the hyperlink for more details on urine dipstick analysis. In many situations, dipsticks give false positive or false negative results. MLT should know the possible reasons false results. The table below lists the reasons for false positive or false negative test results while using dipstick urine analysis methodology.

Able to perform some common laboratory tests

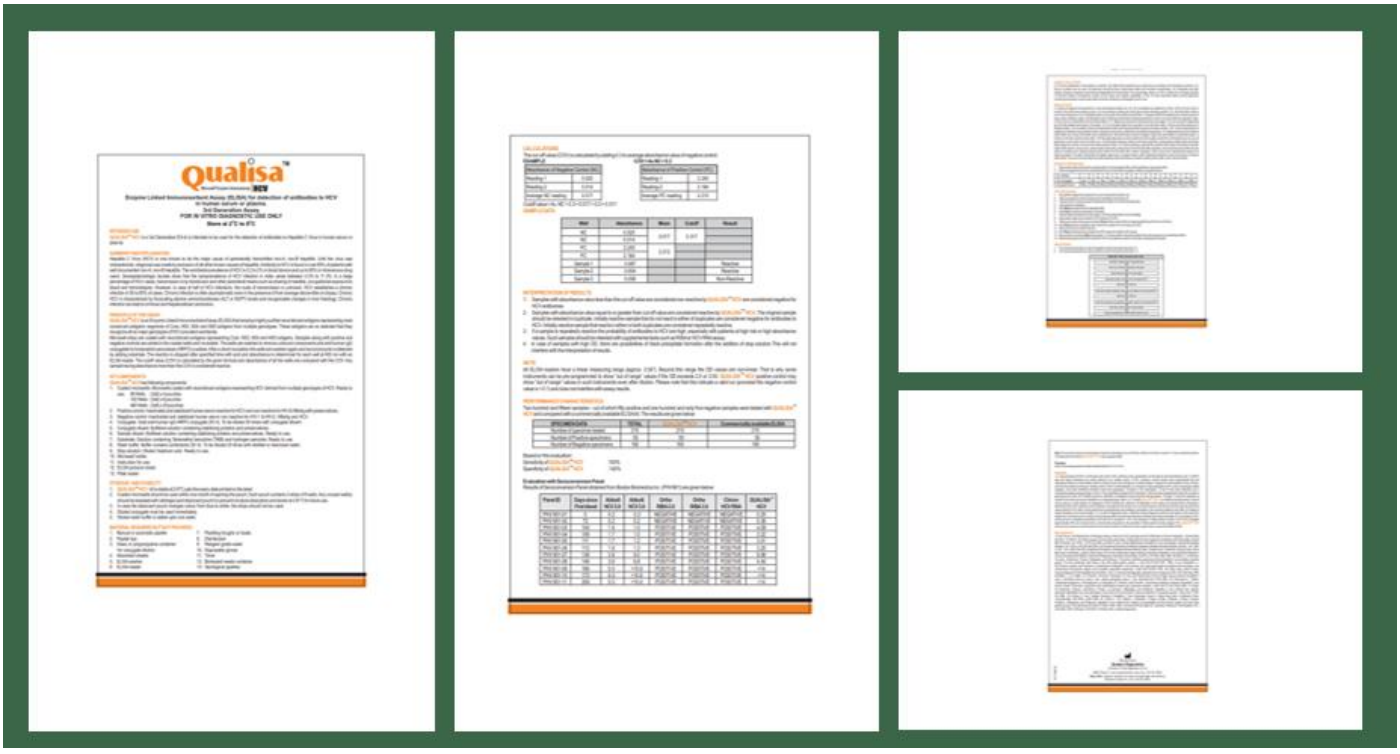
Most of the tests these days are done using semi or fully automated auto analyzers, and the manufacturers provide testing SOPs. The SOPs must be followed while performing the tests.

Diagnostic Kit-based Tests:

Certain tests are done using commercially available test kits. These kits contain all material required for the test, including reagents, slides, mixers, etc. These tests appear easy, but the chances of mistakes are high, particularly with perishable kits that need cold chain maintenance. If the kit remains on the workbench at ambient temperature for 3-4 hours, its efficacy is reduced.

Essential is the pack insert, which is placed inside the kit pack. These pack inserts should be read and understood before testing, particularly while using kits from a different manufacturer. This pack inserts describe in detail the application, principle, contents, procedure, preservation conditions, quality controls, result recording, calculations etc., if required, reasons for false positive and false negative results etc. Photo of a pack insert is given below:f

Pack Insert Photo



Some biochemistry tests, like Urine sugar and Protein Analysis, are done manually in many laboratories. Hence, MLTs should know the test's principle, material required, working principle, test procedure, methods of recording the observations and calculations of the test result, limitations of the procedure, and Situations where the test may give false positive and false negative results.

To Perform Point-Of-Care Testing and Assess Results

Point-of-care testing (POCT) refers to tests conducted near or at the patient's location where care or treatment is being provided. These tests yield results quickly, allowing for immediate action to be taken.

Common examples of point-of-care tests include monitoring blood glucose levels and conducting home pregnancy tests. Other frequently performed tests include measuring hemoglobin levels, detecting fecal occult blood, conducting rapid strep tests, and measuring the prothrombin time/international normalized ratio (PT/INR) for individuals taking the anticoagulant warfarin.

The MLT should be competent to perform all the POCTs as per procedure and precautions given in test kit pack inserts.

As mentioned above the analytical phase in Clinical laboratory testing is very important. If not done carefully, the test results may be affected adversely.In this segment, the trainees will learn the following:

Lessons Learnt

- To follow the test procedure/equipment SOPs properly and understand their importance
- To apply the principles of routine microscopy.
- To apply the physical and chemical principles of staining & the quality of staining and initiates corrective action.'
- To assess results identify sources of interference, and initiate corrective action.
- To perform Card-based immunoassay tests and tests using commercially available ready-to-use kits for tests done at PHCs with knowledge of all the precautions affecting the test results.
- To perform manual counting procedures using cell counters etc.
- To Identify and evaluates the morphology of cellular and non-cellular elements in microscopic preparations.
- To differentiate between clinically significant and insignificant findings to the test done at PHC
- To perform point-of-care testing and assess the results

Post analytical Phase

Recording of results

Each patient's test results should be recorded carefully. There are chances of mis recording between samples. Almost all the semi or fully-automated analyzers have the facility of result printing through the attached printer. The printer must be used and the print used for further processing and laboratory records. Reading the results from the analyser screen could lead to wrong recording.

Comparing with control sample results and validation

After recording the results, for validation purposes, 1st step should be to observe the results of control samples processed with the same batch or with individual samples, as the case may be. Senior and authorized personnel should do this step. If positive control is giving positive results and negative control is giving negative results, then the test sample results processed along with controls are considered to be acceptable.

Critical Call Alert

When a patient's test result is in such a zone that there is a risk to the patient's life, it is known as the critical value. Informing his/her physician in time is known as a critical call alert. This can save the patient by the timely intervention of the doctor.

After validation of the test results, note down if any result value is out of normal value and falls in critical value. E.g. if the result value of the blood sugar of a patient is less than 50 mg/dl and the patient is in the danger zone; it should be considered a critical value and should be reported to the concerned physician within 10 minutes or less time so that the patient can be saved

Release of the report after transcript check

The report formats are filled, and the reports are recorded in the laboratory registers or uploaded on the HIS/LIS system. But before release of the report, MLTs should check the following, which is known as transcript check:

a. Does the report entered on the form belong to the same patient (Patient identity)?
b. Whether the report text mentioned on the report is same as what has been reported by the analyzer, or have you recorded it from the manual test?
c. For any other discrepancy

Transcript check is critical as this is also an essential source mixing of report, mistyping, etc.

Adherence to the guidelines for specimen retention, storage, transportation, and disposal

Sometimes after the report's release, the physician feels some incompatibility of the report with symptoms or, for any other reason, requests the laboratory to repeat the test on the same sample. Under the circumstances, the quality management rules say that the clinical samples should be retained for some time after even the release of reports, as mentioned in the laboratory policy. MLTs should be competent to understand and implement the sample retention rules per the policy. He should have the facilities for preserving samples without any harm to their integrity until they are disposed of.

After completion of the retention period, the samples need to be discarded as per existing Biomedical Waste Management rules. He/she must stick to the rules for storing, storing, and disposing of clinical samples.

In spite of very good test results, the end results could be spoiled by improper post-analytical activities just spoil everything if not done properly. After going through this domain, the trainees will learn the following:

Lessons Learnt

- To report results of all types of tests done in PHCs based on manual reporting like microscopy, color change based, strip tests/ card tests etc., meeting quality control criteria.
- To understand the terminology used in test reports as agreed between laboratory professionals and clinicians.
- Will know the importance of referring to any preliminary report of the same test on the same patient
- To check all the reports for their transcript correctness before releasing the report.
- To compare the results with positive and negative controls.
- To identify unexpected or implausible results and takes appropriate action before reporting.
- To record the test results in Laboratory Information System or on registers as appliable
- To keep records/reporting data in safe custody.
- To recognize and act on critical values by timely communicating the critical reports to the concerned physician.
- To release a report within TAT (Turn Around Time).
- To confirm that the Laboratory's report distribution/delivery system is efficient and that the report reaches the clinician or patient confidentially and in time.

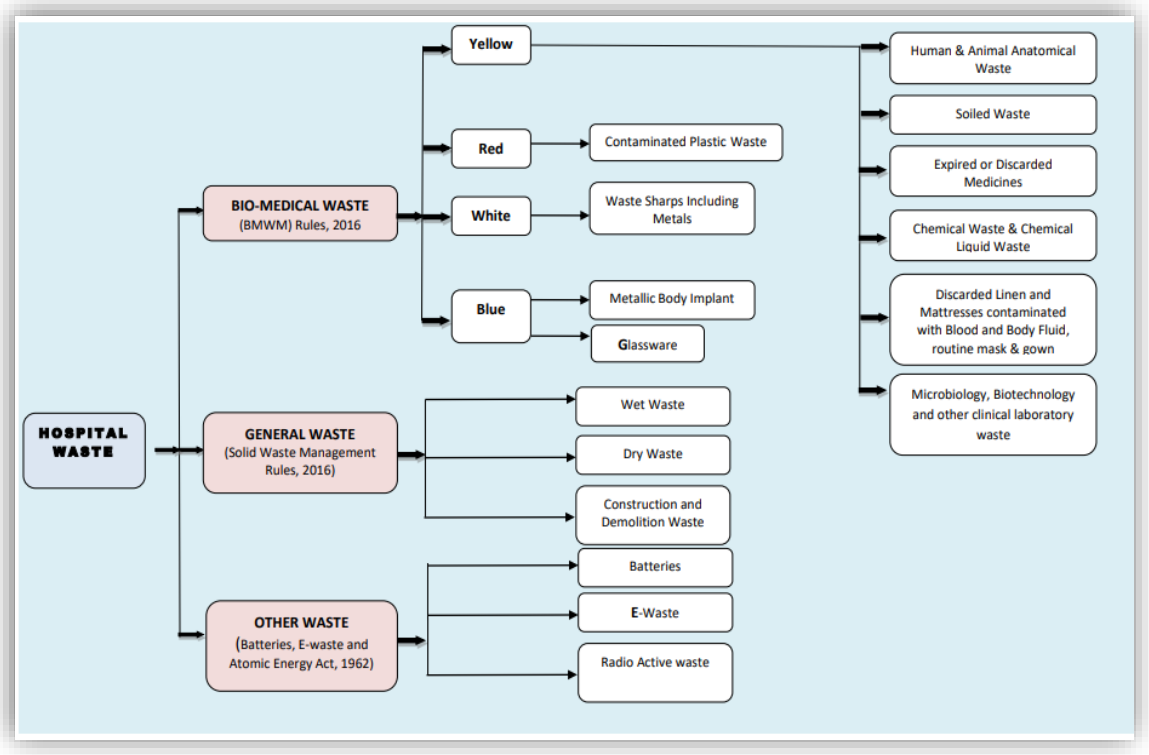
Biomedical Waste Management

To store, handle, transport, and disposal of biological and other hazardous materials according to Bio-Medical Waste Policy

Every hospital, including its laboratory, should have a well-established, well-circulated, and time-to-time updated BMW management policy as Biomedical Waste Management rules framed by Central Pollution Control Board, which all hospital staff should follow.

Follow the following main aspects while dealing with Bio-Medical Waste.

- Segregation at source:
    - It should be the responsibility of laboratory staff to segregate the waste at the time and place of generation in different coloured baskets as per BMW management rules amended from time to time.
    - Following are the waste categories as per BMW management rules:2016



Categorization & Classification of Wastes in Health Care Facilities

Colour-coded baskets should be used as per prevailing BMW rules.

For details, please refer to the Biomedical Waste Management Rules 2016

Disposal of clinical samples and other suspected infectious material: All the clinical samples and suspected infectious waste should be considered infectious and autoclaved in autoclavable bags before handing over

to the BMW disposal agency to ensure that nothing infectious is going out of the laboratory. Here we have shown one example of autoclavable bags. There are so many manufacturers of such bags,.

Liquid waste Management: Many liquids are being used in clinical laboratories which are not biodegradable and should not be drained unless they are neutralized chemically or by some well-established standard dilution methods. For example, Glutaraldehyde is a disinfectant solution used in many hospitals which should always be discarded after neutralizing it with Glycine or with the dilution method:

- Glycine method: Add 30 g of glycine to the waste liquid per 1 liter of the preparation liquid containing 2% glutaraldehyde.
- Dilution method: Dispose of the formulation by diluting 1 liter of the 2% glutaral formulation with 200 liters of water and discarding it (dilution method).

To dispose of chemicals, dyes, reagents, solutions, and used samples after completion of the retention period according to BMW rules, local legislation, and as per Master Safety Data Sheet (MSDS)

All the expired chemicals, used vials etc., should be disposed of as per prevailing BMW rules. Any of the clinical samples should be considered potentially hazardous and should be autoclaved before sending that outside the laboratory to any of the BMW management agencies or the self-BMW management system of the hospital.

As mentioned above, clinical laboratories generate a lot of infectious and non-infectious waste. The trainees will learn the following after going through this domain.

Lessons Learnt

- To store, handle, transport, and disposes of biological and other hazardous materials according to Bio-Medical Waste Policy.
- To understand color coding of hospital/laboratory waste buckets and waste transportation bags
- To handle and disposes of sharps (Bio-Medical Waste Policy).
- To implement the concept of "Segregation at source" while generating the waste.
- To use disinfection and sterilization methods to disinfect materials used.
- To select disinfectant as required.
- To test the efficacy of Sterilizers.
- To minimize potential hazards related to disinfection/sterilization methods.
- To understand and implement all laboratory waste management rules and requirements as per Central Pollution Control Board (CPCB) guidelines

Laboratory Associated Infections: Safety and Controls

General and Specific Safety Precautions of Clinical Laboratory

The clinical laboratory receives several clinical samples. As per bio-hazards/Bio-medical waste management rules, all human samples should be considered infectious and dealt with full safety precautions. The Measures are employed when handling bio-hazardous materials to avoid infecting oneself or others or contaminating the product and the environment. Clinical laboratories use a significant number of chemicals that may be hazardous, like acids, alcohols etc., which might pose a threat/risk of accidents. The laboratory staff is prone to be harmed by infectious and non-infectious incidents, which may harm laboratory staff, the laboratory environment, or even samples. Hence, MLTs must be competent to deal with such incidents or accidents. They must be vigilant about such incidents, associated hazards, and safety precautions to avoid harm to themselves, the sample, and the environment.

A few essential and specific aspects in which MLTs must be competent are:

To understand the routes of Laboratory Acquired infections and to apply the standard precautions to prevent the spread of infection as per organization requirements / local rules and other rules as applicable.

A competent MLT should know about the sources and routes of laboratory-acquired infections in his/her laboratory. They should evaluate the possible risks while working in the laboratory. A few routes of infection in a clinical laboratory are as follows:

- **Inoculation**
    - Introduction of organisms into the eye by
        - splashing or
        - rubbing with contaminated fingers
    - Injection through the skin by
        - needle stick injury
        - Incision by sharp instruments / broken glass
    - Rubbing the skin with Contaminated fingers/instruments
- **Ingestion**
    - Through oral route by
        - Licking, Sucking, Accidental swallowing of infected material
    - Specially mouth pipetting of cultures / infected material
        - Cotton wool plug at the upper end – not itself safe
        - The finger to control the pipette – may itself be contaminated
    - Contaminated fingers / writing instruments/licking labels
    - During eating, drinking, and smoking in the lab
- **Inhalation**
    - Through the respiratory route by
        - Breathing in an infected material
    - Aerosol – the cloud of tiny droplets of liquid in the air
        - Usually contains many droplets of 0.1 mm
        - Droplets – dry rapidly to become solid residues called droplet nuclei (1-20 µm). It may remain in the air – for hours.
    - Invisible aerosols are generated by any action that breaks down the continuity of the liquid surface and even during centrifugation.
- **Conventional and other air currents**
    - Disperse aerosols and dust
        - Inside laboratory
        - Into adjacent rooms
        - The particles >5 µm

- Deposited into nose and throat
- Smaller particles Can reach deep into the lungs

How and under what circumstances laboratory infections may occur in clinical laboratories?

- Needle stick injury during use or disassembly or re-capping
  - Others – improperly disposed syringe
- Aerosols generation
  - Vibrating needle on withdrawal from a vein or culture
  - Splashing – during forceful injection of contents
  - Centrifugation of contaminated materials like live cultures or samples from a patient with infectious disease
- The skin, clothing, or bench – contaminated by
  - Spillage of contaminated material
  - Leakage from the syringe
  - Backflow of inoculum after injection into an animal
- Pipetting
  - Infected material – ingested during mouth pipetting
  - Disseminated to surrounding – last drop blow off
  - Auto pipettes/ Micropipettes – produce aerosols
  - Infective material stabbed into the skin – broken glass pipettes
- Inoculation loop - aerosol formation
  - Vibrating inoculation loop
    - Especially if longer than 4-5 cm
  - Flaming of wet loop/cooling (Sizzling) of the hot loop
  - Mixing a slide agglutination test
  - Making a smear for staining
- Petri-dishes
  - The water of condensation on agar or lid
    - Contaminated
    - Split on to fingers or on to the bench
- Mixing, shaking/homogenizing/centrifugation
  - Aerosols in a closed container
    - spread on opening
    - Imperfect seal

- Ultracentrifuge: Exhaust through HEPA
- Gross environmental contamination
  - On breakage during these procedures
- Transportation of samples
  - Inadequate closing and packing of samples
    - Contaminate wrappings and forms/documents/ packing inserts
    - Outside of samples – may be contaminated
  - All samples should be treated as potentially hazardous upon receiving them in the lab.
- Non-Infectious Incidents
  - Acid spills
  - Fire accidents due to alcohol spills or electrical short circuiting etc.

Use Of Personal Protective Equipment Appropriately, E.G., Gloves, Gowns, Masks, Face Shields, Aprons

A critical safety precaution is to wear Personnel Protective Equipment (PPE) while working in a clinical laboratory. These include gloves, gowns, masks, face shields, aprons, shoe covers etc.

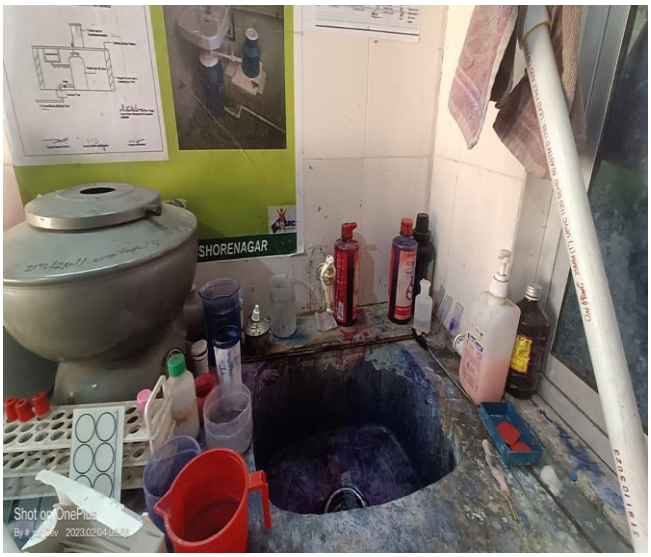
Keeping laboratory hygiene and infection control practices / Policies intact and followed.

Every laboratory should have a written policy on laboratory hygiene and Laboratory Infections Control Practices, and all the staff members should follow them.

Laboratory hygiene:

The dirt is the primary source of contamination and poses a threat not only to infectious hazards but also to allergic complications to the staff. The laboratory should be cleaned at least once daily and as required

thereafter. The cardboard boxes attract dust, hence should not be allowed inside the laboratory working area. Those should be limited to the inventory area, separate from the laboratory.



The laboratory should have sufficient space which can be routinely cleaned. Under any circumstances, cleaning is a must. See the below-given photo:

Use of laboratory safety devices like biosafety cabinets, safety pipetting devices, safety containers, and carriers etc., to minimize possible dangers from Clinical specimens

Clinical specimens/samples may be potentially infectious, hence extreme care should be taken while dealing with them.

After collection, clinical samples should be transported in closed containers, preferably insulated boxes, and not exposed to open environment. If samples are to be transported under cold conditions, the cool packs or dry ice can be used in insulated boxes.

- The sample containing boxes should be transported with the utmost care, avoiding any chance of an accident or fall off.
- Only micropipettes/autopipette devices should be used. Mouth pipetting should never be done.
- The infectious samples should be dealt with in a safety cabinet.
- The patients should not be allowed inside the Tuberculosis laboratory or stand or sit in front of the Tuberculosis laboratory.
- MLTs should wear proper PPE in the laboratory. If working with tuberculosis samples, they should wear N95 masks.

Using recommended or self-validated methods of disinfection and sterilization to disinfect materials to be used or to be disposed of

Sterilization is a process of freeing an article from viable microbes, including their spores. A satisfactory sterilization process kills >10^6 organisms with high-degree resistant spores. It is usually executed by physical methods like Heat (Autoclave etc.), filtration and radiation etc.

The MLT should know about different sterilization methods, their working principle, applications, and limitations so that he/she may choose the right method of sterilization for the article to be sterilized. Normally sterilization is done at 121°C, achieved at a pressure of 15 lbs/in^2 for 15-20 minutes. Some culture media which contain carbohydrates are sterilized at 110 or 115°C. But sterilization of infectious biomedical waste is done at 134°C for 18-30 minutes.

Participants should also understand

- **Sterilization hold time (Holding period):**
  - Exposure time at an effective temperature to ensure complete sterilization
  - Derived from the Thermal death time of *B. Stearothermophilus* (R > *Cl. Tetani*)
- **Heat penetration time:**
  - The load takes time to attain the required temperature.
- **Importance of air removal from an autoclave during the process of autoclaving:**
  - Air is heavier than steam and a bad conductor of heat
  - Forms a layer at the lower level – decreased temperature

For efficacy testing of sterilizers, please refer to the Equipment section.

Disinfection is the process of freeing an article or a surface from harmful organisms. The satisfactory disinfection process is that which destroys all vegetative microorganisms. It is usually done for heat-labile articles or where heat cannot be applied and is executed by chemical methods. Disinfection is of three types.

- Low level – Kills all vegetative organisms and may or may not kill some spores.
- Medium level - Kills all vegetative organisms and Mycobacterium tuberculosis and some spores
- High level - Kills all vegetative organisms and Mycobacterium tuberculosis and most of the spores

For a good application of disinfection, it is necessary to understand the process, particularly the factors that negatively affect the process of disinfection.

Factors affecting disinfection and General rules to perform disinfection:

- Generally toxic
    - may damage or irritate skin, conjunctiva, and mucus membranes
    - Be careful while using
    - Avoid contact with irritant or toxic conc.
    - Gloves should be worn
- Antimicrobial spectrum varies
    - Choose disinfectant according to the purpose
    - A written disinfection policy should be there to specify the use
- Working concentration of disinfectant
    - Accurate in-use concentration should be made
    - Low conc. (ineffective), High (irritant)
    - Appropriate concentration as recommended by the manufacturer.
- Deterioration of working dilution
    - Some disinfectants deteriorate when diluted with water, like chlorine-generating compounds or other oxidative agents like H_2O_2
    - Deteriorate by microbes / organic material to which they are applied
    - Hence be changed in time
- Proper immersion of the article
    - Disinfectants may not kill even highly susceptible organisms if the article is not immersed properly.
    - The disinfectant must not be overloaded
    - All surfaces should come in contact

- Should be immersed properly

- Contact time
    - For disinfection, a proper contact period should be given for process completion as the manufacturer recommends.
    - Effective time varies with
        - Microbial load
        - Presence of organic material
        - Temperature
        - pH
        - Nature of the exposed surface
        - Presence of resistant bacteria /spores
- Other reasons for Inactivation
    - Presence of soap and detergent of opposite polarity
    - Presence of
        - Cork
        - Cellulose
        - Cotton rubber and
        - Other discard materials

Hence, disinfectants should not be mixed with other disinfectants or cleaning solutions.

Selecting a disinfectant to be used in the laboratory

The MLT should understand many factors related to disinfection. Only then he/she will be able to choose the right disinfectant for the target article.

The disinfectant should be chosen as per the situation and application. A few examples are as follows:

- **Laboratory discard Jars:** After dilution, the hypochlorite or bleach has only 6-8 hours of effectivity. After dilution, they immediately start deteriorating and lose their chlorine concentration. In many laboratories, the solution for discarding Jars is not changed frequently, deteriorates, and does not stay effective for the required time. If these solutions are used, they should always be made fresh to kill the contaminating microbes effectively.

If there is a constraint to make fresh solutions everyday, many disinfectants are available that do not deteriorate significantly, even 2-3 days after dilution. Those are Quaternary ammonium compound-based disinfectants. For these disinfectants , MLT should be competent in performing the in-use test for

standardizing the change time of disinfectant in a particular laboratory set-up. This needs to be done because different laboratories have different waste load to be discarded in discard jars.

- Many labs use Glutaraldehyde containing disinfectants on floors or for internal fogging. Glutaraldehyde is highly toxic and may cause many complications and should never be used in open-air systems for floors or aerial disinfection. For floors, the Quaternary ammonium compound-based disinfectants are advised. Arial fogging or fumigation is not recommended, as all high-risk areas need AHUs fitted with HEPA filters. However, if required for aerial disinfection, H_2O_2-based disinfectants are the preferred options.

To document all incidents related to safety and personal injury like Needle Stick Injury

Sometimes, unwanted incidents occur in the laboratory which pose a threat to the personnel, environment, or to clinical samples e.g

- Needle stick injuries
- Breakage of sharps
- Sample or any other infectious material spills
- Acid or any other hazardous chemical spill.

, Any such incident , should be recorded electronically if the lab has the LIS system in place or on hard copy registers. No incident should be ignored and left un-recorded and not-informed to higher authorities. Even a small needle stick injury can transfer Blood born infection, particularly HIV, Hepatitis B&C etc. Hence, it should be reported and recorded for further treatment if required. The designated specialist in the hospital should have all the records, and he/she should proceed as per the Infection control policy of the organization. The spills should be taken care of as per standard rules. In all such incidents, corrective action, root cause analysis, and preventive actions should be taken immediately so that such incidents do not happen again or at least be minimized.

Hence, MLTs should take care of the above-mentioned factors and others if noticed by him/her for safety in the laboratory.

To follow protocols for care following exposure to blood or other body fluids as required

If any person exposed to blood or any other body fluid, the actions should be taken immediately for the safety of the exposed personnel as per laboratory policy. If required, medical attention should be extended to him/her.

Safe Spill Management

Clinical laboratories deal with many chemical and infectious materials, which always pose a threat to laboratory professionals.

Chemical Spill Management

In laboratories, a variety of dangerous substances are used in different amounts. To ensure a safe response to chemical spills, it is important to have a thorough plan in place. Dealing with a chemical spill should only be done by knowledgeable and experienced experts. It is essential to have spill kits readily available, containing instructions, absorbents, reactants, and protective equipment for cleaning up minor spills. Minor chemical spills can be safely managed by laboratory staff without needing assistance from safety or emergency personnel. However, any other chemical spills are considered major and require additional support.

Minor Chemical Spill

- Notify individuals in the immediate vicinity about the spill.
- Put on personal protective equipment (PPE) such as safety goggles, gloves, and a long-sleeve lab coat.
- Avoid inhaling any vapors from the spill.
- Minor spills that are contained to a small area usually do not require respiratory protection.
- Use the appropriate neutralizer and absorbent for inorganic acids and bases.
- Collect the remaining residue, place it in a container, and dispose of it according to the chemical waste policy.
- Use the appropriate kit or absorb the spill with materials like vermiculite or dry sand for spills involving other chemicals.
- Collect the remaining residue, place it in a container, and dispose of it as chemical waste.
- Clean the spill area with water.

Major Chemical Spill

- Attend to individuals who are injured or contaminated and remove them from further exposure.
- Notify everyone in the laboratory to evacuate.
- If the spilled material is flammable, turn off any sources of ignition or heat.
- Call the designated Emergency Response number for chemical spills.
- Close the doors to the area affected by the spill.

- Have someone knowledgeable about the incident and the laboratory assist the emergency personnel.

Biological/Infectious spills

Spillage of blood and body fluids is known as Biological/infectious spills. There may be three types of spills:

- Spot spill
- Minor spill
- Major spill

Spot Spill: The spot is immediately wiped out using a cotton gauze soaked with 70% alcohol, and the spot is cleaned. Discard the contaminated material in the yellow basket and wash your hands thoroughly.

Minor spill: The spills which spread over an area of less than ten centimeters is considered to be minor spill which may be dealt with as follows:

- Collect cleaning material, equipment etc.
- Wear Proper PPE, including an apron, eye shield, face mask, and gloves.
- Cover the area of the spill with a paper towel or any other absorbent cloth.
- Cover the area with 10% bleach or 1% Sodium hypochlorite properly.
- Wait for 30 minutes.
- Collect the contaminated material using a scraper and put it in a yellow bag.
- Wipe the area with a cleaning paper towel or cloth and remove any residual blood.
- Mop the spill area and surrounding area with Sodium hypochlorite again.
- Wipe with warm water and any detergent to further clean the area.
- Wash your hand thoroughly.

Major Spill: The spills that spread over an area of ten centimetres or more is considered major spill. The following are the steps to deal with it.

- Evacuate the laboratory as in major spills; aerosol formation is possible.
- Wait for any aerosols to settle
- Collect cleaning material, equipment etc.
- Wear Proper PPE, including an apron, eye shield, face mask, and gloves.
- Cover the area of the spill with a paper towel or any other absorbent cloth.
- Cover the area with 10% bleach or 1% Sodium hypochlorite properly.
- Wait for 30 minutes.

- Collect the contaminated material using a scraper and put it in a yellow bag.
- Wipe the area with a cleaning paper towel or cloth and remove any residual blood.
- Mop the spill area and surrounding area with Sodium hypochlorite again.
- Wipe with warm water and any detergent to further clean the area.
- Wash your hand thoroughly.

Biological Spill Management Kit

Every clinical Laboratory should have a readily available spill management kit.

Components of Spill Management kit

- Gloves, face mask, eye shield and shoe covers etc. (Required PPE)
- Absorbent cloth/towel or napkin papers
- Cleaning mops
- Bleaching powder or Sodium chloride
- Any other specific material as per requirement

Diagrammatic Representation of Biological Spill Management

To place appropriate signs wherever and whenever required, e.g., hazardous, flammable, restricted entry, containment zone etc.

As per the situation, the signages below should be placed for personnel awareness. Training for understanding the importance of this signage should be given to the lab staff. These signages are helpful, particularly for less educated personnel like sanitation attendants etc.

Sharp Cuts and Abrasions Management

Broken glass can cause lacerations when it comes into contact with the skin, similar to sharp objects like needles and razors. While the mishandling of glassware primarily poses a risk to the user, anyone near broken glass is also at risk of injury. Therefore, everyone must wear their personal protective equipment (PPE) as a precautionary measure. The likelihood of being injured by broken or breaking glass is considered 'LIKELY,' and the potential severity of injury from incidents involving pressurized or vacuum glass apparatus is 'SEVERE TO EXTREME.' By implementing safety controls and ensuring the use of PPE, the potential risks associated with handling glassware can be minimized.

Please refer to the table below for a detailed understanding of Possible Risks and Safety Controls.

Type of Glassware Injury	Safety Control Recommendations for Risk Reduction	Example Process/Activities	Potential Risk
Injury from imploding glass or flying glass. (Vacuum apparatus)	• Tape vacuum flasks and vacuum desiccators with electrical tape. • Keep cryogenic vacuum flasks wrapped with Nylon or other polymer based plastic mesh (view example). • Wrap glass desiccators with friction or electrical tape in a grid pattern, leaving the contents visible; this will guard against flying glass in case of implosion. • Wear a face shield or safety glasses with side shields, appropriate gloves, and lab coat. • Educate researchers in vacuum techniques. *Consider other factors that could possibly reduce risks, such as: Use low-intensity vacuum devices, use smaller flasks, assure vacuum is released at the vacuum pump before removing vacuum flask/glassware, and make sure glassware under vacuum is not located where it could be bumped or struck.*	Working with vacuum apparatus.	HIGH
Cuts when forcing glass tubing or pipettes onto rubber stoppers	• Use glycerin as a lubricant. • Wear cut-resistant gloves and safety glasses with side shields.	Working with glass tubes or Pasteur pipettes.	Moderate Plus
Cuts caused by breaking inlet/outlet stems from reflux condensers or desiccator stems	• Dip the end of Tygon tubing into a solvent such as acetone and gently insert onto glass stems. • Wear cut-resistant gloves, safety glasses with side shields, and lab coat.	Assembling/disassembling condensers, or holding the stem to open or close desiccators.	Moderate
Chemical or other hazardous material exposure caused by sharps injuries from	• Develop and implement hazardous chemical or hazardous material-specific SOPs.	Working with or transferring toxic chemicals in reaction flasks, separatory funnels, etc.	Moderate

contaminated glassware	• Work within a fume hood or appropriate engineering control. • Wear gloves, safety glasses with side shields, and lab coat. • Wear a face shield or safety goggles as appropriate for the specific experiment.		
Broken or breaking glass during laboratory work/process (Injury from slipping glass)	• Check the glassware to ensure it is free of cracks, flaws or scratches. • Wear slip-resistant and/or cut-resistant gloves, safety glasses with side shields, and lab coat when washing glassware. • Wear safety glasses with side shield for transporting glassware outside the lab. • Wear a face shield or safety goggles as appropriate for the specific experiment.	Washing lab glassware, transporting to or from drying ovens, autoclaves, etc.	Low
Breaking glass ampules	Use ampoule breakers or triangular file to score the glass as appropriate.	Working with pharmaceutical and analytical standards or other sealed chemical ampoules.	Low
Inserting the inlet of an auto-pipetting device onto a glass pipette	• Check for defective pipettes with cracked or chipped ends.	Dispensing liquid reagents in labs.	Low
Cutting glass tubes	• Purchase commercially available aspirator flasks with prefabricated stopped/Pyrex glass tubing assemblies. • Use a triangular file or diamond pencil for scoring/scratching a glass tube or visit your local glass shop for assistance (335-1337)	Preparing aspirator/vacuum flasks filtration equipment	Low

Good space:

Working in the clinical laboratory with space constraint not only invite accidents, infection spread, and contamination but also reduces the competency of the laboratory personnel.

The laboratory space should be sufficient with proper shelves to place the reagents, kits, chemicals etc. The instrument room should be separate. Working shelves and the inventory room should be separate.

Cardboard boxes should be allowed only up to the inventory room, not the working area and instrument room. The personnel should have enough space for free movement.


To maintain hand hygiene by washing hands before and after patient contact and/or after any activity likely to cause contamination.

Hands are the most frequent transporters of microbes from the source to the host. Hence all the MLTs and other health and Allied health professionals should develop a habit of frequent hand washing. A few points to understand are given below:

Our skin, particularly our hands, has two types of microbes.

Resident flora: These stay on our hands almost permanently as commensals and are not harmful to us. Shown in photo with **green color**

Transient flora: These are the microbes that adhere to our hands from the contaminated surface on touching that and are shown here in **red color**.

These microbes may be harmful and are transmitted from the host to the patient, causing infection.


WHO and CDC say proper and timely hand washing reduces more than 80% of hospital-acquired infections. Similar principles are applied while working in a laboratory. Hence proper hand washing should be encouraged.

Opportunities to wash hands:

- Before and after every episode of direct patient contact
- After any activity or contact that potentially results in hands becoming contaminated
    - Blood
    - body fluids
    - Secretions
    - Excretions
    - contaminated items
    - Before wearing gloves
    - After glove removal
    - Between contact with different patients

To use alcohol-based hand sanitizers, if justified, e.g., soap and water are not available or any other such barrier

Sometimes, personnel do not wash their hands timely due to some barriers or excuses. Some of them are as follows:

- Hand washing facilities not available
- Need hand cleaning very frequently
- Hands remain wet for longer periods
- Need a sterile towel every time

Under these circumstances, alcohol-based hand sanitizer may be encouraged. While working in the clinical laboratory, the MLTs and other staff may be exposed to infectious material and other accidents with sharp and non-sharp articles. Hence, after going through this domain, the trainees will learn as follows:

Lessons Learnt

- To demonstrate general and specific Safety Principles of Clinical Laboratory.
- To use personal protective equipment appropriately, e.g., gloves, gowns, masks, face shields, aprons
- To know about laboratory hygiene and infection control practices / Policy
- To minimize possible dangers from biological specimens, laboratory supplies, and equipment (Biosafety)
- To use laboratory safety devices, e.g., laminar flow systems, biosafety cabinets, safety pipetting devices, safety containers, and carriers, eyewashes

- To apply measures in response to laboratory accidents/incidents (blood spills and centrifuge tube breakage during the process)
- To document all incidents related to safety and personal injury, like Needle Stick Injury.
- To apply the standard precautions to prevent the spread of infection as per organization requirements / local rules and other rules as appliable.
- To minimize contamination of materials, equipment, instruments, and environment by aerosol and splatter.
- To follow protocols for care following exposure to blood or other body fluids as required.
- To place appropriate signs wherever and whenever required, e.g., hazardous, flammable, restricted entry, containment zone etc.
- To maintain hand hygiene by washing hands before and after patient contact and/or after any activity likely to cause contamination.
- To use alcohol-based hand sanitizers, if justified, e.g., soap and water are not available or any other such barrier.
- To deal with cuts and abrasions with waterproof dressings and changes as necessary.
- To demarcate and maintain clean and contaminated zones in all health care work.

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TRANSFORMATION PLATFORM

C1 Block, Plot No 4, ISID Campus
Institutional Area II, Vasant Kunj, New Delhi 110070

+91-11-6132 6500 | +91-11-6132 6599
www.hstp.org.in | @HSTPIndia