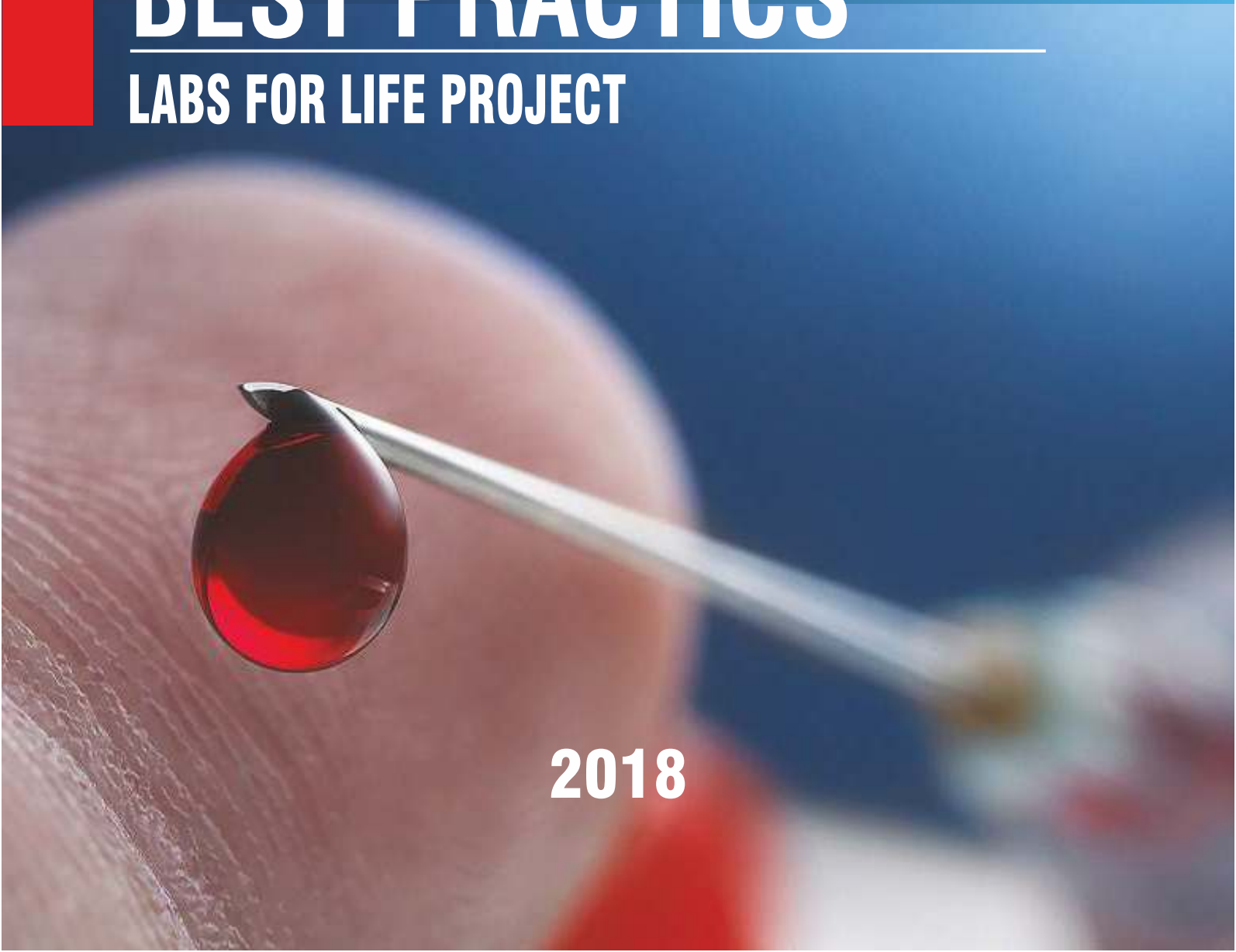
सत्यमेव जयते

Ministry of Health & Family Welfare
Government of India

TRAINING MODULE ON SAMPLE COLLECTION AND PRE-ANALYTICAL BEST PRACTICS

LABS FOR LIFE PROJECT



2018

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Government of India

Labs for Life is a partnership project of the Ministry of Health and Family Welfare (MoHFW) and the U.S. Centers for Disease Control and Prevention (CDC), to strengthen the public health laboratories in the country through lab workforce development and implementation of Quality Management System.

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सत्यमेव जयते

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Government of India
Ministry of Health & Family Welfare
National AIDS Control Organisation

Foreword

For the successful functioning of any organization, it is important not only to improve its core activities, but also streamline the supporting processes. Often these support services form the link between the organization and its users, creating value for the provider and improving the satisfaction of the user. In the medical laboratory, the pre- analytical processes constitute the "front end" for the customer, where the lab meets its user.

Moreover, laboratories are complex entities with clearly demarcated pre-analytical, analytical requirements, across several disciplines. Each area poses its own challenges. Laboratory standardization is the norm of the day, standard guidelines being available for each area and each activity. These need to be incorporated into the public health lab management practices.

To make a beginning in this journey towards quality, we have to begin at the pre analytical phase. Sample collection and handling influences manifold the overall laboratory performance and patient satisfaction. As a beginning to the series on different aspects of laboratory quality management system, this training module has been developed by a team of experts in the field, towards understanding Sample Collection and Pre-analytical Best Practices.

This module provides a very comprehensive knowledge in all pre-analytical areas and I hope it proves useful to all those who are utilizing this.

(Sanjeeva Kumar)

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Preface

A laboratory report is only as good as the sample that it receives. Sample collection irregularities, apart from producing incorrect reports, also lead to repeat test requests inconveniencing the patient and adding to laboratory costs. The cycle of laboratory improvement must begin by addressing the quality of sample to make an overall impact.

The role of the laboratory commences right after the clinician prescribes a test. The pre-analytical processes encompass patient registration, patient preparation, sample collection, transport, storage and processing. Good pre-analytical practices also touch upon the safety of patients and laboratory staff along with safe disposal of bio-medical waste generated in the process. This module has been modeled on the ISO standard and gives information about the prescribed standards for test requisition, patient communication, consent requirements, sampling procedure, sample acceptance or rejection and sample traceability. It also describes different phlebotomy equipment including closed collection devices, different types of sample containers, pre- and post-exposure prophylaxis for accidental exposure to blood, and bio-medical waste management guidelines. Finally, the need to set appropriate quality indicators and monitor the performance in the pre-analytical activities of the laboratory has been discussed.

I hope this module will help laboratories to address the deficiencies in this area of working and improve the reliability and efficiency of their services

(Alok Saxena)

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Message

Facility management and safety are inseparable components of a laboratory. All organizations, whether public or private, use buildings, assets, and services to support their activities. Facility management effectively coordinates these assets and services, thereby helps in optimizing costs, maximizing performance and improving return on investments.

Laboratories are facilities, which are usually open 24 hours a day, seven days a week and perform a complex range of services. Therefore, it is important that laboratory workspace and facilities are designed to provide quality results and ensure the safety of staff, patients, and the community. The workflow should be streamlined to avoid overlapping of tasks and incompatible activities should be separated from the main work area.

Safety in a laboratory has been overlooked for long. "Safety First" should be inculcated in the work culture of every laboratory and in the mind of every healthcare worker. A safe work environment is essential not only for the wellbeing of staff but it also instills faith and confidence in the patients towards the laboratory.

This module has been created to communicate the essential elements of laboratory facility management and safety. Biosafety, fire safety, and equipment safety are some of the topics that have been discussed in detail in this module; the module also elaborates the procedures to develop an effective safety program for a laboratory. Enhancing the overall safety standards of laboratories will improve the experience of all stakeholders. We hope this document will be a useful guide for ready reference in times of need.

(Dr. Naresh Goel)

Acknowledgement

The comprehensive training module on Sample Collection and Pre-analytical Best practices has been developed through coordinated and concerted efforts of various organizations, individuals and professional bodies. We are grateful for the time devoted and the expertise provided by them.

Dr Anu George, Technical Manager and the entire Labs for Life team are acknowledged for their hard work in providing direction and structure to this manual. We are also thankful for the support of subject area experts from Christian Medical College (CMC), Vellore, and National Centre for Disease Control (NCDC), and National Health Systems Resource Centre (NHSRC) who jointly reviewed the technical content and provided valuable technical inputs.

Apart from resources generously shared by Becton Dickinson (BD), the material in this training module has been compiled from multiple open sources such as Textbooks, Manuals, National and International Guidelines, Review Articles and related Websites.

Our sincere thanks to the U.S. Centers for Disease Control and Prevention-Division of Global HIV/TB (CDC/DGHT), India Office, its implementing partner Christian Medical Association of India(CMAI) and Becton and Dickenson, collaborating partner for Labs for Life project for providing technical assistance, guidance and support in the preparation of this module.

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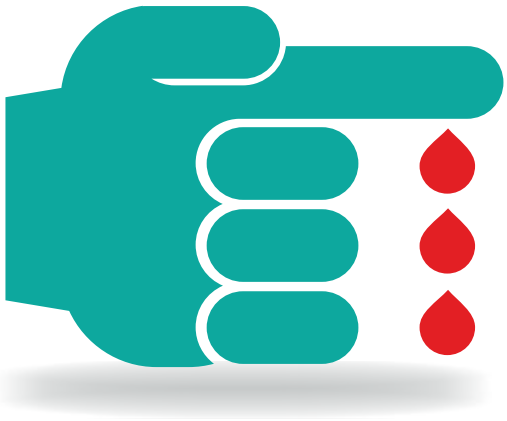


Importance of Sample Collection, Sample Transportation Referral How to Use this Manual

Background

Several studies indicate that an increasing number of clinical decisions are based on results generated by laboratories. Therefore laboratories play a very important role in ensuring favorable patient outcome. The accuracy of lab test results is directly proportional to the quality of the sample collected, hence the importance of sample collection cannot be overemphasized. Each step in the process of biological sample collection affects the quality of the specimen and is thus important for preventing pre-analytical laboratory errors. Studies have revealed that approximately 68% of laboratory errors are associated with the pre-analytical phase of laboratory processes. Besides these errors, sample collections also pose risks for health workers through needle-stick injury and exposure to body fluids leading to transmission of diseases.

In conditions where lab tests cannot be conducted at the site of the collection, biological specimens need to be transported. There is a need for safe and efficient transportation process as to reduce chances of exposure to all involved.

Moreover, the contaminated sharps and other material generated at the time of specimen collection need to be disposed of properly so as to ensure complete safety to the healthcare workers, patients and community. After the completion of analysis, emphasis shined be placed on the propose discarding of biological samples and the collection equipment.

Various policies and procedures are established for the collection, transportation and discarding of biological samples to ensure integrity of the samples and safety of the patient, healthcare worker and the environment. These are based on recommendations from ISO–(ISO15189; 2012),Centers for Disease Control and Prevention (CDC), Clinical Laboratory Standards Institute (CLSI),OSHA and WHO guidelines

Purpose and Objectives

This manual has been prepared for training in best practices for sample collection, receipt, processing, packaging transportation and safe disposal of biological samples.

This manual will help to:

- Understand the best practices as defined under the standard guidelines
- Understand the responsibility of management and technical departments in pre-analytical processes
- Identify the non-conforming events with regard to specimen integrity and associated patient and health care worker safety
- Educate and promote safe practices and reduce blood-borne pathogen exposure and transmission
- Improve patient confidence and comfort and to avoid and manage associated complications
- Improve the quality of laboratory test results by assuring specimen integrity
- Understand the guidelines for correct specimen referral, transportation and storage for accurate test results
- Understand the guidelines for safe disposal and bio-medical waste management.

Target Audience

The manual is intended for the Master Trainers participating in the preliminary phase or the Training of Trainers (TOT). This manual may be used for further training within the institutions for:

- People who perform or supervise specimen collection, receipt, referral, transportation, processing, testing, storage and disposal in public sector hospitals, community clinics and other health-care facilities, including those involved in home-based care
- Health care trainers and educators
- Management/administrative officials and other stakeholders.

Materials and Methods

An orientation of the contents of this manual will be given to the master trainers in the training of trainers. Hand-outs and training aids will be provided for onsite training. The onsite training by these trainers will also be observed & monitored for their efficiency, practicality and accuracy.

How to Use This Manual

There are twelve chapters in this module. A brief about each chapter is given below:

Chapter 1: General Information

This section contains information about the quality assurance in pre-analytical processes. There are certain key which require constant monitoring and action.

The responsibilities of the management and technical personnel towards pre-analytical activities are also defined in this section.

Chapter 2: Patient Registration

This chapter describes the need for registration as a service contract, the need for test requisitions, patient tracking and informed consent.

Chapter 3: Patient Preparation for various tests

ISO 15189:2012 under 5.4.4 mandates full information to the patients in the pre-collection activities. A few such activities are suggested under this heading. However, the lab is required to assess the pre-collection activities pertinent to their methods of testing and accordingly develop a protocol.

Chapter 4: Sample Collection - Blood

Blood sample collections have been described under venous, capillary and arterial. Under each heading the collection equipment, methodology and complications, are listed and wherever relevant, special techniques have been defined.

Chapter 5: Specimen other than blood

Samples other than blood have been described as

a) Patient collected samples,
b) Microbiology samples which may be collected by the physicians or laboratory staff
c) Exclusively physician collected samples.

Under each heading is a description of collection activities, special storage and transportation requirements

Chapter 6: Field sampling in Epidemics

This chapter explains the standard guidelines for sample collection from the field during epidemics.

Chapter 7: Sample Receipt (Accessioning), Rejection/Acceptance and Processing

This section explains the process of receiving samples in the laboratory, both in-house and from outside the laboratory, applying the sample rejection criteria, and processing of samples before sending them into the laboratory.

Chapter 8: Sample Referral and Transportation

This chapter talks about the need for referral, choice of referral labs, preparation of samples for transportation and the process of safe transportation.

Chapter 9: Sample Storage

This chapter explains the need, mechanisms and optimal storage time for different samples.

Chapter 10: Biosafety and Infection Control in Pre-analytical processes

This chapter lists out the diseases that have been seen to be transmitted through inappropriate pre-analytical practices and mechanisms to prevent them.

Chapter 11: Biomedical Waste Management

This chapter lists out the types of bio hazardous wastes, purpose for waste management and the guidelines for safe waste disposal.

Chapter 12: Monitoring and Evaluation

This chapter explains how to assign and monitor quality indicators to offer surveillance of management of pre-analytical processes and associated adverse events, enabling the laboratory to develop quality objectives and continual improvement mechanisms.

Chapter 1

General Information

1.1 Learning Objectives

At the end of this chapter learners should be able to define:

a) Elements of Quality Assurance in Pre-analytical Processes

b) Management/administrative responsibilities

c) Responsibilities of the technical staff

They should also be able to make use of signage and other relevant instructions

1.2 Elements of Quality Assurance in Pre-analytical Processes

Table: 1.1 Elements of Quality Assurance in Pre-analytical Processes

Element	Notes
Education and training	Education and training is necessary for all staff carrying out pre-analytical activities. It should include an understanding of anatomy, awareness of the risks from blood/ biological sample exposure and the consequences of inappropriate sample collection technique leading to poor infection prevention and control.
Standard operating procedures (SOPs)	SOPs are required for each step or procedure. They should be written, read and understood the by designated laboratory staff and be readily available to all health care workers involved in sample collection.
Correct identification of the patient	Identification should be through matching with the laboratory Test Request Form "TRF" with a minimum of two positive patient identifiers After samples have been taken from a patient, a system of identification and tracking is essential to ensure that the sample is correctly matched with the result and with the patient or donor.
Traceability of sample	Unique numbering/labelling of samples that can be traced back to the correct patients
The quality of the Sample	The sample should be maintained in proper condition that would result in necessary preservation in order to ensure that the quality of the results is satisfactory.
Safe transportation and storage	Proper packaging, safe transportation and appropriate storage of biological samples will ensure improved - quality of test results and overall safety.
An incident reporting system	A system is required for reporting all adverse events. A log book or register should be prepared with accurate details of the incident, possible causes and management (corrective and preventive action) of adverse events.
Immunization of laboratory staff with HBV vaccine and Anti HBs testing to ensure protective titer.	Documentation of status of HBV immunization is mandatory

1.3 Management Responsibilities

Management responsibilities include provision of space and equipment, record keeping and infection control facilities.

- The management should provide separate primary sample collection facility in the laboratory.
- The design of the laboratory should ensure high efficiency of its operations, safety of its occupants and minimize the risk of injury and occupational illness.
- The Laboratory's instructions for pre-collection, collection and post-collection activities should be displayed in the respective areas.
- Provision should be made in patient privacy, comfort and needs (e.g. toilet facility, availability of drinking water) and a waiting area for accompanying persons (e.g. guardian or interpreter) during collection.
- A bed/couch should be available in the collection area to manage patients in the event of vasovagal attacks.
- First aid kit should be also in place to take care of complications during blood sample collection.
- Appropriate facilities should be provided for safe storage of patient samples to avoid deterioration, loss or damage during pre-examination activities and during handling, preparation and storage.
- There should be dedicated space for sample reception
- Appropriate policy and procedures must be in place for accepting verbal requests for tests and requests for additional examinations on the same primary sample.
- Sufficient supplies of personal protective equipment and infection control aids should be made available, which include hand-hygiene materials (soap and water or alcohol rub), well-fitting gloves, aprons, single-use disposable needles, and syringes or lancing devices these should be in sufficient numbers to ensure that each patient has a sterile needle and syringe or equivalent for each blood sampling, as well as sharp boxes, and discard containers for proper segregation of waste at source. Expired items should not be used.
- The management is also encouraged to make available sufficient laboratory sample collection tubes to prevent unsafe practices (e.g. decanting blood to recycled laboratory tubes). Several safety-engineered devices are available in the market; such devices, reduce exposure to blood and prevent accidental injuries. However, the use of such devices should be accompanied by other infection prevention and control practices, and training and sensitization of the end user. It is very important for them to ensure that they are able to reduce incidence of needle stick injury in the limited resources available.
- Availability of post-exposure prophylaxis: In the event of an accidental exposure, the incident should be recorded in a register. There should be documented procedure of the process to be followed in case of any reported incident. The staff should be aware of the

institutional protocol and should know whom to contact in such a condition. Post Exposure Prophylaxis (PEP) protocol should be followed for those who undergo accidental exposure. PEP can help to avert sero-conversion in HIV and hepatitis B infections. Complete Hepatitis B immunization (0, 1 and 6) should be provided to all health care workers (including cleaners and waste handlers), either upon entry into health-care services or as part of PEP. The antibody titer of those immunized should be timely evaluated as per the institutional guidelines.

1.4 Technical Responsibilities and Role of Phlebotomists

- An SOP/manual/handbook for sample collection should be available at all collection sites.
- All phlebotomists/nurses/doctors should be trained in SOP and should have easy access to the Sample Collection Manual.
- A system for reporting of non-conforming events should be made available to the collection staff.
- Periodic training, regular supervision and competency evaluation of the collection staff should be done and records should be maintained.
- Correct identification of patient must be done by confirming at least two unique identifiers and demographic details.
- Informed consent from patients and maintenance of confidentiality should be ensured, wherever it is mandatory.
- Ensuring that correct specimen collection method is used and specimen is mixed immediately after collection as appropriate and labeled immediately.
- Where primary sample collection is done in the laboratory/primary sample collection area; the technician is responsible for the entire process.
- In case the samples are withdrawn outside the lab, the staff is responsible for checking the suitability of the sample for testing (and filling up the lab requisition form) and applying the sample rejection criteria.
- Ensuring that the integrity of the sample is maintained till analysis. Any verbal test request for examination should be followed by written request by the concerned physician/or as defined in the lab's SOP.
- In emergency situations, consent might not be possible (if required); under these circumstances it should be acceptable to carry out necessary procedures, provided they are in the patient's best interest.
- In case of sample collection done in the ward by doctors/nurses, it should be ensured that they have duly-filled requisition form prior to sample collection. This should be emphasized by fegnent reminders (in the form of circulars/letters) for the same.
- Patients in a hospital bed/ICU may be given an option to refuse sample collection.
- Avoidance of contaminated phlebotomy equipment
- Tourniquets are a potential source of methicillin-resistant *Staphylococcus aureus* (MRSA), with up to 25% of tourniquets contaminated through lack of hand hygiene on the part of the phlebotomist or reuse of contaminated tourniquets. In addition, reusable finger-prick

devices and related point-of-care testing devices (e.g. glucometers) contaminated with blood have been implicated in outbreaks of hepatitis B. To avoid contamination, any common-use items, such as glucometers, should be visibly clean before use on a patient, and single-use items should not be reused

1.5 Signage in Sample Collection Area

The following signage are encouraged in the waiting areas (preferably in local language)

- Room no. /names, path of flow for patients, enquiry room (in case feasible).
- The name(s) and contact information of the In-Charge/laboratory director and /or other laboratory staff.
- List of services available.(Directory of services available)
- List of services excluded.
- List of free services
- Patient preparation instructions
- Instructions for Patient-Collected samples
- Rate list, if applicable.
- Turnaround time.
- Directions to Toilets, drinking water, fire exits, use of fire extinguishers etc.
- Any other instructions specific for the laboratory.

The following signages are encouraged in the sample collection areas (preferably in local language)

- Restricted entry
- The bio-hazard sign (See Annexure).
- Order of draw of samples
- Instructions for Bio medical waste management
- Instructions for any special collections
- Spill management
- Preparation of 1% Sodium Hypochlorite Solution
- NSI with contact details for PEP (Counseling, testing, drugs, follow up)
- Any other instructions specific for laboratory.

Chapter 2

Patient Registration

2.1 Learning Objectives

At the end of this chapter, the learners should be able to define:

a) The meaning of Service Agreements and review of service agreements

b) The concept of Test request forms (TRF) generated by the referring physician and those generated by the labs.

c) System of Lab Numbering and unique identification

d) The need for informed consent

2.2 Service Agreements and Review of Service Agreements

The ISO 15189:2102 says that laboratories must have documented procedures for the establishment and review of agreements for providing medical laboratory services.

Each request accepted by the laboratory for examination(s) is to be considered a service agreement.

Agreements to provide medical laboratory services shall take into account the request, the examination and the report. The agreement shall specify the information needed on the request to ensure appropriate examination and result interpretation.

The following conditions shall be met when the laboratory enters into an agreement to provide medical laboratory services.

- The requirements of the customers and users, and of the provider of the laboratory services, including the examination processes to be used, shall be defined, documented and understood.
- The laboratory shall have the capability and resources to meet the requirements.
- Laboratory personnel shall have the skills and expertise necessary for the performance of the intended examinations.
- Examination procedures selected shall be appropriate and able to meet the customers' needs Customers and users shall be informed of deviations from the agreement that impact upon the examination results
- Reference shall be made to any work referred by the laboratory to a referral laboratory or consultant.
- Reviews of agreements to provide medical laboratory services shall include all aspects of the agreement. Records of these reviews shall include any changes to the agreement and any pertinent discussions.

When an agreement needs to be amended after laboratory services have commenced, the same agreement review process shall be repeated and any amendments shall be communicated to all affected parties.

2.3 Test Request Forms

Test requests are generated by the prescribing doctor and are processed further in the lab by including unique identification numbers, time of collection and billing details if required. This process may be defined by the lab suitably. The format of the request form (e.g. electronic or paper) and the manner in which requests are to be communicated to the laboratory should be determined in discussion with the users of laboratory services.

2.3.1 Test Request Forms: Prescriptions

Every request for tests should be accompanied by a written form which includes patient identification, including gender, date of birth, and the location/contact details of the patient, and a unique identifier; name or other unique identifier of clinician, healthcare provider, or other person legally authorized to request examinations or use medical

information, together with the destination for the report and contact details, type of primary sample and, where relevant, the anatomic site of origin; examinations requested, clinically relevant information about the patient. Information needed for examination performance and results interpretation must be asked for and may include the patient's ancestry, family history, travel and exposure history, communicable diseases, history of medication and other clinically relevant information

The laboratory must have a documented procedure concerning verbal requests for examinations that includes providing confirmation by request form or electronic equivalent within a given time.

The laboratory must cooperate with users or their representatives in clarifying the user's request.

2.3.2. Test Request Form: Lab

Data entry should begin as soon as registration number is assigned; to the patient in OPD collections or at the time of the test ordering/sample receipt from the ward and referrals.

If a request form (TRF) generated at this point of registration, it must ensure that all the data enumerated in ISO 15189:2012 are incorporated. Alternatively, the physician generated prescription may be used for identification and tracking of the sample. Whichever is the system chosen, the process must be a suitably defined.

Financial information for billing purposes, financial audit, resource management and utilization reviews may also be collected. The patient should be aware of the information collected and the purpose for which it is collected.

Date and time of primary sample collection in OPD collections; date and time of sample receipt in the event of ward or referral samples must be noted.

2.4 Lab Numbering and Unique Identification

A unique identifier is an important tool for managing information, and careful thought should be given for how best to assign identifiers to patients and samples within the information management system.

2.4.1 Patient identifiers:

Sometimes hospitalized patients are assigned a unique identifier upon admission, to be used for the duration of the hospital stay. A patient may get a new number each time he/she is seen or admitted. In other settings, the unique identifier may be assigned to the patient on a more permanent basis, to be used each time the patient has any health care.

2.4.2 Sample identifiers:

Laboratories need to assign unique identifiers to patient samples so they can be tracked throughout the laboratory. Unique identification includes an alpha and/or numerical identifier such as a hospital number, or personal number like Aadhar number.

The method for generating and assigning unique identifiers within an information management system will depend on many factors. Some commercially available computer systems for laboratories have a numbering system built in to the software. Laboratories using paper-based systems will need to establish their own system.

An example of a simple system for generating unique identifiers is as follows. Consider using a number consisting of the year, the month, the day, and a four digit number: YYMMDDXXXX. At the beginning of each day, the last four digits start with the number 0001.

For example, the number 1508250047 can be read 15 08 25 0047, and it would represent: sample #47, received on August 25, 2015.

To avoid confusion or mix-up of samples use the sample's full identifying number throughout the laboratory. At a minimum, the unique number will need to be used on all aliquots of the sample, on the request form, the laboratory register or log, and the result sheet.

Whatever system a laboratory chooses, unique identifiers should be used to eliminate confusion and mix-up of samples, and make samples and information easier to find.

2.5 Informed Consent

All procedures carried out on a patient need the informed consent of the patient. For most routine laboratory procedures, consent can be inferred when the patient presents himself or herself at a laboratory with a request form and willingly submits to the usual collecting procedure, for example, venipuncture. Patients in a hospital bed should normally be given the opportunity to refuse.

Special procedures, including more invasive procedures, or those with an increased risk of complications to the procedure, will need a more detailed explanation and, in some cases, written consent.

In emergency situations, consent might not be possible; under these circumstances it is acceptable to carry out necessary procedures, provided they are in the patient's best interest.

Annexure A:

Sample request form, Sample Informed consent form

Chapter 3

Pre Collection Guidelines Patients Preparations for Various Tests

3.1 Learning Objectives

At the end of this chapter learners should be able to understand:

a) The prerequisites of each test so as to minimize pre-analytical errors
b) The current guidelines for Glucose Loading tests
c) How to allow patient participation and support for specific tests to ensure best results

Note: The word “specimen” and “sample” are interchangeable

3.2 Physiological Variability

Majority of the tests do not require any specific patient preparation. However, fasting morning specimen is preferred for many of tests performed on serum, plasma or whole blood. Several changes in values can happen due to physiological variables. Examples of physiological variations include food intake, circadian rhythms, travel, sweating, prolonged bed rest, posture, environmental factors and seasonal influences. A few precautions are suggested to ensure best results.

Some Examples of physiological variability

Electrolytes, S. Proteins:

- Alteration of values can occur due to sweating. The patient should be advised to sit down till sweating subsides before sample is collected

Urea and urates

- High protein intake can increase values of urea and urates. The patient should be advised to have a balanced diet a night before to avoid error.

3.3 Fasting Tests

a) Fasting Plasma Glucose:

- Fasting period of 8-10 hours is required

b) Lipid Profile:

- 10-12 hours of fasting is required.

c) Parathyroid Hormone:

- 12 hours fasting is required (Depending on the analytical requirements)

3.4 Glucose Loading Tests

a) Post Prandial Plasma Glucose:

Adequate breakfast is to be eaten after giving collection of fasting sample. Blood specimen is collected 2 hours after food intake. If the patient is diabetic and is on medication, he/she should take the medicines, as usual.

b) Preparation for Glucose Tolerance Tests:

- Perform GTT in the morning after 3 days of unrestricted diet and activity
- Perform only in ambulatory subjects
- 10-16 hours of fasting recommended.
- The tests should begin between 7- 9 AM
- The subject should seated in between sample collection
- Smoking is to be avoided in between sample collection

c) Oral Glucose Tolerance Test:

The two samples are taken at following intervals

- Fasting. Then, 75gm of glucose in 300ml of water is given to the patient. The load should be ingested within 5 minutes.
- 2 Hour, after glucose load.

d) Screening and Diagnosis of GDM at 24-28 Weeks of Gestation

2 Methods:

i) 1 Step Strategy (75 grams load):
Fasting (> 8 hours fast), followed by 2 post load samples; 1 hour and 2 hours

ii) 2 Step Strategy:

a) 1st step is screening with 50 grams (non-fasting), sample drawn at 1 hour

b) 2nd step (if > 140 mg/dL in screening) use a 100 gram load after fasting and followed by 3 samples at 1 hour intervals:

Note: Please refer current ADA guideline

e) Pediatric Glucose Tolerance Test:

- 1.75 grams of glucose/Kg body weight to a maximum of 75 grams in 300ml of water is given to the patient, the load should be ingested within 5 minutes.

3.5 Sequential Specimen Collections

- For some hormonal studies, specimens are collected at specific timings e.g. Cortisol levels. The blood specimens are collected at 8.00am and 4.00pm. The time of blood specimen collection has clinical significance and hence must be clearly mentioned on the sample collection tube.
- For diagnosis of endocrine disorders: Because of the episodic, circadian and cyclic variations in the secretions of gonadotropin, a pooled sample is preferred which are drawn thrice at an interval of 20 minutes. Prolactin sample is best withdrawn 3 to 4 hours after the subject has awakened.

3.6 Serial Monitoring (Monitoring a patient over time for specific conditions)

Done for various tests e.g. different tumor markers, antibody titers for follow-up of the patient after treatment e.g. Widal test, HIV,VDRL.

3.7 Semen Analysis

- A period of 3-5 days of abstinence is required before semen analysis

3.8 Timed Collections

- Instruct the patient to discard the first urine passed in the morning and note down the exact time (e.g. 8.00am). From this time onwards collect all subsequent urine samples in the container provided. Collection should be continued till same time next day (e.g. 8.00 AM). The 8 AM sample voided also should be collected.
- The patient should return the 24 hours urine sample container within 1 hr. to the lab.

Chapter 4

Sample Collection: Blood (Venous, Capillary, Arterial)

4.1 Learning Objectives

At the end of this chapter, the learners should be able to define:

a) Different equipment used in blood collection; venous, capillary and arterial
b) Preferred sites for performing collections; venous, capillary and arterial
c) Standard methods, complications and management of venous, capillary and arterial blood collection
d) Special techniques in blood collection

4.2 Phlebotomy (Venous Blood Collection)

4.2.1 Blood Sampling Systems: Equipment (Open and Closed systems available)

a) Closed Systems:

Closed or evacuated systems for blood sampling are preferable because they have been proven to be safer than open systems and provide better specimen quality. The added advantage of using closed system is that blood comes directly in contact with the anticoagulant avoiding many pre-analytical issues such as micro-clot formation.

Figure 1: Closed System, Needles & Holder

Figure 2: Evacuated tubes

The use of evacuated blood collection systems reduces the risk of direct exposure to blood and has made it easier to take multiple samples from a single venipuncture. Although vacuum extraction systems are safe, training and skill is required for their correct use.

- Double-ended needles are available in several recommended gauge sizes.
- The non-patient end of the needle is covered by rubber sleeves and it is screwed into the barrel (also known as the tube holder, evacuated tube holder).
- A thread separates the two ends of the needle; it is where the holder is screwed into place.
- Regular needles/flashback needles are available. While using regular needles; it is difficult to make sure that vein has been entered while performing venipuncture. Whereas, while using needles with flashback, the phlebotomist gets flash of blood in hub of needle the moment it enters the vein. One of the reasons why the needle and syringe practice is prevalent is because it gives the operator the confidence of being in the vein when they spot blood on the transparent needle hub. While this is not possible in regular needles used in evacuated system, the user gets a similar flash while using needles with flashback feature.

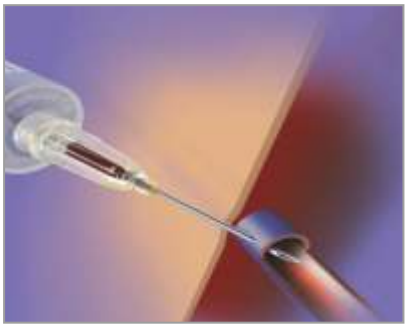


Figure 3: Flashback Needles

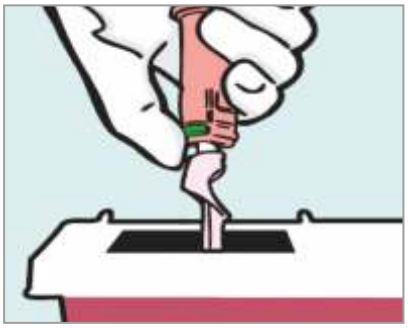


Figure: 4 Built-In Mechanism to release the needle

- The holder holds the sample collection tube in place and protects the phlebotomist from direct contact with blood.
- As the sample tube is under vacuum, once the needle is in the vein, the tube is pressed on to the non-patient end of the needle in the holder and blood is drawn automatically into the sample tube by vacuum until the required amount is collected.
- The system comes complete with needle, holder and the laboratory sample tubes with appropriate additives and color-coded caps depending on different types of additives / samples.
- Discard the needle, holder and syringe as a single entity where possible.
- If there is a need to reuse the holder (NOT RECOMMENDED), use a one-hand scoop technique (see below) to cover the sharp end of the needle and thus to safely remove the needle from the holder.

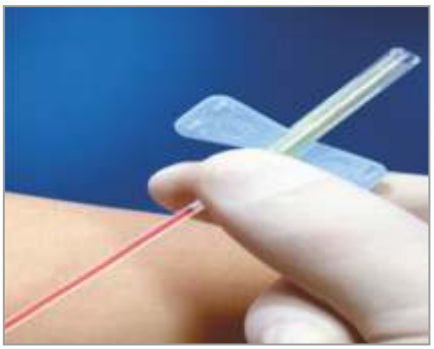


Figure 5: Patient end of needle retracted into the safety shield

- Alternatively, one could use holders with a built-in mechanism to release the needle by pressing a button. Thus eliminating need for re-capping the needle.
- Some systems have a mechanism that can be activated once the needle has been used; the mechanism either retracts the used needle into the barrel or snaps it shut or employs a safety shield on the needle. Vacuum systems may also be used with a winged butterfly needle and Luer-lock connectors.
- The sharps container must be within arm's reach and clearly visible, to ensure safe disposal of sharps. The instructions for appropriate use and disposal can be displayed in the form of flowcharts on the working station.

Winged Blood Collection set with Luer Adapter

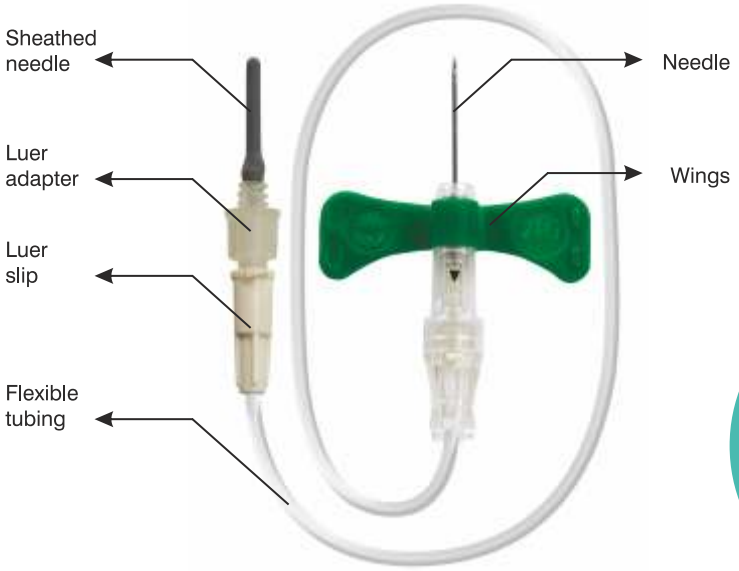


Figure 6: Winged Butterfly with Luer-lock Connectors

Note: Store all tubes at 4-25ºC unless otherwise noted on the package label. Extreme temperatures can reduce the effectiveness of the tubes and cause abstract results. Always remember to rotate your stock.)

b) Open System

Open systems include hypodermic needle and syringes and sample container. Sometimes winged steel needles attached to a syringe may be used in-place of hypodermic needles. The use of a hypodermic needle and syringe is the most common means of blood sampling.

Assembling of Needle and Syringe

To use a needle and syringe system:

- Open the packaging of the hypodermic needle from the hub end (back of the needle), keeping it capped;
- Open the sterile packaging of the syringe from the plunger end (back of the syringe), keeping the nozzle protected in the packaging;
- Carefully remove the syringe from the packaging and insert the nozzle of the syringe firmly into the exposed hub of the capped hypodermic needle;
- Leave the needle and syringe in place until ready for use.

c) Needle-Choice of Gauge

If the needle is too large for the vein for which it is intended, it will tear the vein and cause bleeding (haematoma).If the needle is too large Blood enters tube faster and forcefully and may results in hemolysis. If the needle is too small, it will damage the blood cells during sampling, and laboratory tests that require whole blood cells, and tests such as K+, Bilirubin, LDH etc. that get affected due to hemolysis, will be invalid.

Table 4.1: Recommended needle gauge, length and device for phlebotomy procedures for different age groups

Patient Population			
Needle Gauge	**Adult**	**Pediatric, elderly, small veins**	**Neonatal**
21	√(1–1.5 inch or 2.54 cm)	**NA**	**NA**
22	√(1 inch or 2.54 cm)	√(1 inch or 2.54 cm)	**NA**
23	√(1–1.5 inch or 2.54 cm)	(Winged set butterfly]; 0.5 inch or 0.75 cm)	(Winged et [butterfly]; 0.5 inch or 0.75 cm)

4.2.2 Procedure

- **Assemble the required materials:**
  - Closed collection systems /disposable sterile plastic syringe 2ml, 5ml, 10ml, 20ml and preferably 22 (black) or 23 (blue) gauge & winged butterfly needles for children. Either regular syringes or evacuated blood systems may be used
  - Tourniquet, Disposable well-fitting gloves, masks, guaze, disinfectant (e.g. 1% sodium hypochlorite), 70% isopropyl alcohol swabs, betadine, dry cotton, Bandaid, lab-coat, hand sanitizer.
  - Blood culture bottles

- Citrate tube for coagulation tests (Light blue cap) (0.109M 3.2% buffered sodium citrate)
- Plain tube without clot activator (Red cap)
- Gel tube (yellow top) with clot activators and Gel separator
- Green tubes containing Sodium/lithium heparin for cytogenetics and plasma based chemistry assays.
- EDTA tube containing K2 EDTA (Lavender cap) (K3 and K2 EDTA are available. K2 EDTA is preferred as it produces less artifacts in analysis)
- Grey tubes containing fluoride for glucose
- Black tubes for ESR
- Specimen Storage racks
- Needle destroyer
- Puncture proof containers for sharps
- Biomedical Waste color coded bins
- Phlebotomy chair.
- Bar-code generator (If available)/Permanent Marker/Pen
- Computer with Laboratory Information System access (If available)

- **Pre-collection verification, use of PPE:**
  - Approach the patient in a calm, confident and professional manner. The phlebotomist must gain the patient's confidence. Assure the patient that, although the venipuncture will be slightly painful, it will be short in duration and necessary for the diagnosis and treatment of their health care problem. Answer any other patient query related to laboratory procedure.
  - Confirm the identity of the patient with the TRF.
  - Wearing gloves is mandatory before sample collection
  - Wear lab coat and masks (especially in TB patients). Closed shoes are preferable to avoid accidental needle stick injury.
  - Cover all injured/exposed wounds/cuts with a bandage before proceeding for sample collection.

- **Determine the best site for venipuncture:**

  Common Sites:

  A. Ante cubital Area:

  - Median cubital vein: It is the most preferred site as it is large, well anchored, least painful and least likely to bruise.
  - Cephalic vein is next preferred site. It is large but not well anchored and may be more painful than Median cubital
  - Basilic vein is the third choice. It is generally large, easy to palpate but not well anchored. It involves the greatest risk as it lies near brachial artery and the median nerve either of each can be easily punctured.

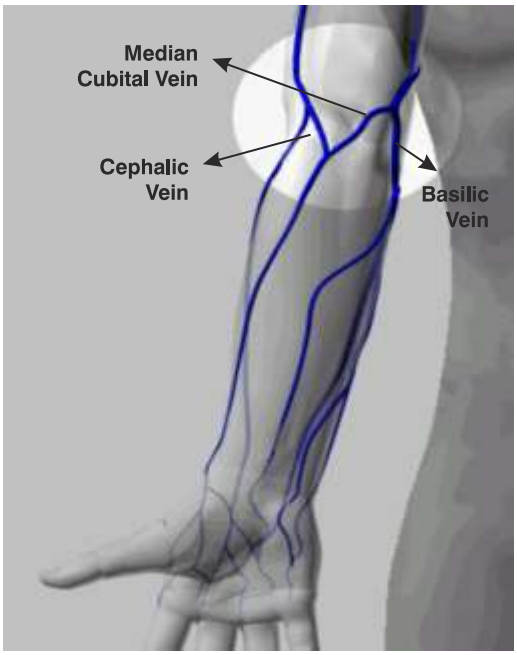


Figure 7: Median Cubital Veins

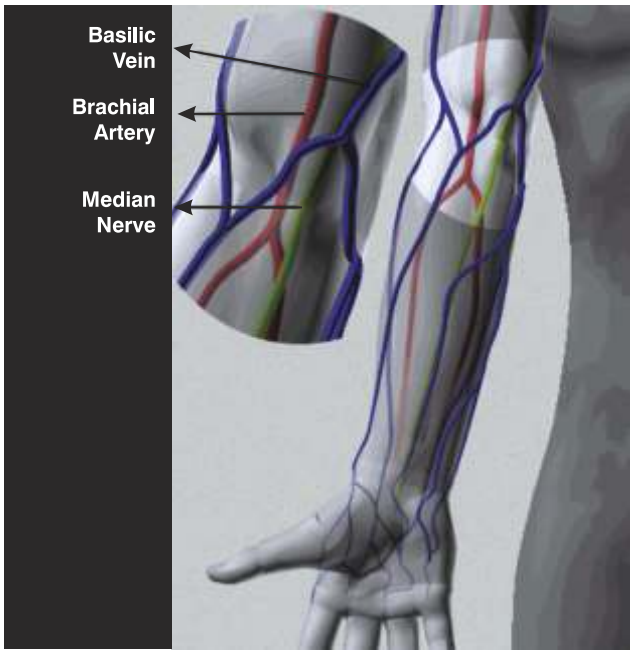


Figure 8: Artery, Nerve and Veins

B. Dorsum of the hand:

- The veins on the dorsum of the hand have a greater tendency to bleed. In fat persons however, it may be difficult to access an ante-cubital veins; in them the veins in the dorsum of the hand are easier to reach.

C. Alternate Sites:

Phlebotomists will not collect except under special circumstances from:

- The arm or hand from the side of a mastectomy unless all other sites have been ruled out, and only with written physician/nurse permission.
- Limbs with indwelling artificial access devices unless all other sites have been ruled out, and only with written permission of primary care physician

D. Unacceptable Sites

- Phlebotomists will not collect from: Fistulas, shunts, arterial lines, or locks. Arteries, femoral vein, varicose veins and the palmar region (inside) of wrist.

- **Venipuncture**
  - Labeling of tubes: May be done prior to or after collection, as per the policy of the institution. The label should be clearly written with the information required by the laboratory, which is typically the patient's first and last names, medical identification number, date of birth/ age, and the date and time when the blood was drawn.
  - After the patient is made comfortable and seated on a chair, ensure proper positioning of the patients' arm.
  - Apply the tourniquet with 3 inch clearance above the planned puncture site. Ideally, the tourniquet should not be applied for longer than one minute at a time. Leaving the tourniquet applied for an excessive period of time (>1min) may cause localized stasis, formation of a partial filtrate of blood and hemoconcentration (small molecules e.g. electrolytes move from capillary to interstitial space). These may result in erroneously high values for all protein-based analytes, packed cell volumes, and other cellular

elements. For coagulation studies, avoid using tourniquet or apply it for < 1minute). Platelet activation may lead to coagulation testing errors.

- Cleanse Venipuncture site with 70% isopropyl alcohol swabs or sterile swabs using a circular motion from the center to the periphery. Never do the reverse.
- Allow the area to dry before venipuncture. The puncture will be painful if alcohol is not dried. Haemolysis is also a possibility if alcohol gets into the sample. Do not re-palpate the venipucture site once it has been cleansed with alcohol.
- If the site must be touched after preparation, the phlebotomist must disinfect the gloves used for palpation. (Ref: Bailey & Scott's Diagnostic Microbiology)
- Alert the patient before the venipuncture. Ask the patient to close their fist but do not allow the patient to pump or clench their fist - tell them to relax. Make sure that patients arm is in downward position to prevent back flow /reflux.
- Anchor the vein and smoothly insert the needle with bevel up at 15 – 30 degrees angle.
- In case of difficulty in location of vein, phlebotomist will tell patient about the same. Lab should have a policy to record multiple punctures. Multiple punctures can decrease patient confidence and satisfaction.
- A clean, sterile needle must be used for each new collection attempt. NEVER re-prick a patient using the same needle.
- Release the tourniquet as soon as the blood begins to flow.
- Avoid excessive negative pressure. No attempt should be made to withdraw blood faster than the vein is filling. This will lead to insufficient quantity of sample, hemolysis and contributes to vein and patient trauma.

- **Needle Removal**
  - The tourniquet must be fully released and patient's hand opened and relaxed before the needle is removed.
  - Hold dry cotton/ gauze pack over the site. Gently and quickly remove the needle from the arm as soon as the needle is removed (and not before); pressure must be applied to the site to avoid leakage of blood and hematoma formation.
  - Ask the patient to keep the pressure on the gauze with thumb of the opposite hand till the bleeding stops.
  - The arm is to be kept straight and not bent at the elbow. In some patients bleeding may continue longer than other patients.
  - Instruct the patients to discard the blood-stained swab in yellow bag (kept at convenient distance) before leaving.
  - Provide with the relevant information in case the patient asks the phlebotomist.

- **Delivering the blood**

  Deliver carefully into respective tubes as per requirement for tests ordered and in proper order according to guidelines given below (Annexure Order of Draw).

  a) **Vacuum Extraction Systems**

  When using evacuated system where blood directly flows into the tubes, ensure that the tubes are kept in-place till the blood flow completely stops (vacuum is exhausted).This would ensure collection of appropriate volume of blood without the need the open the cap.

b) Syringe and Needle, with evacuated tubes

Opening the cap of an evacuated tube increases the risk of compromising the integrity of the primary sample

If a syringe is used, it is recommended to place the tube into a rack before filling the tube. To prevent needle-stick injuries, use one hand technique to puncture the tubes with the needle to fill the tube under vacuum. Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of haemolysis.

Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate coloured stopper. DO NOT remove the stopper because it will release the vacuum.

As an alternative, there are safer devices available to transfer blood from syringes to evacuated tubes. These are called Blood transfer device. This device allows user to attach syringe barrel to attach on one end while the other end is mounted on a holder to allow transfer of blood to evacuated tubes without need to open the cap. These devices may be considered as a better and safer alternative.

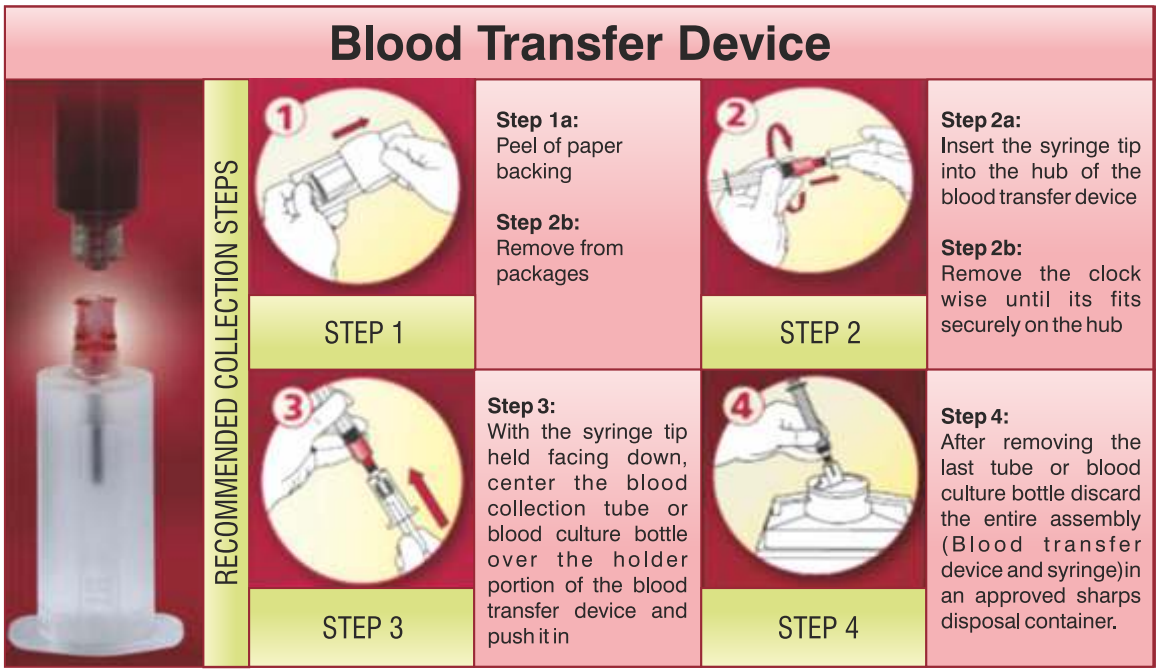


Figure 9: Blood Transferring Device

c) Syringe and Needle with non-vacuum tubes

If non-vacuum tubes are used, remove and discard the needle in sharps container (the needle destruction procedure given below may be followed first) remove the cap of the tube and transfer blood extremely slowly into the tube minimizing the pressure and velocity to risk of haemolysis. Ensure that appropriate volume of blood is transferred to each tube. Ensure that there is no froth formation during the transfer process. Phlebotomist should never push the plunger too hard in order to avoid froth formation / hemolysis and also risk of aerosol formation (since the tube is open). Do not overfill. Replace the container cap.

- **Inversions**
  - Immediately after collection invert the tubes containing additives for the required number of times (as specified by the manufacturers/institute guidelines.)

Table: 4.2 Order of draw and mixing requirements
(Always follow your facility's instructions for order of draw) Annexure: B

Order of use	Type of tube/usual colour	Additive	Mode of action	Uses	Inversions
1	Blood culture bottle	Liquid broth culture medium	Preserves viability of microorganisms	Microbiology – aerobes, anaerobes, fungi	–
2	Light Blue Top Coagulation tube	Sodium citrate	Forms calcium salts to remove calcium	Coagulation tests Requires full draw	3-5
3	Red Top Clot activator	Clot activator	Blood clots, and the serum is separated by centrifugation	Chemistries, immunology and serology, blood bank (cross-match)	4-5
4	Yellow Top Serum separator tube	None	Contains a gel at the bottom to separate blood from serum on centrifugation	Chemistries, immunology and serology	4-5
5	Dark Green Top Heparin	Sodium heparin or lithium heparin	Inactivates thrombin and thromboplastin	For lithium level use sodium heparin, For ammonia level use either	8-10
6	Purple Top EDTA	EDTA	Forms calcium salts to remove calcium	Haematology, Blood Bank (cross-match) requires full draw	8-10
7	Pale Yellow Top Blood tube	Acid-citrate-dextrose (ACD, ACDA or ACDB)	Complement inactivation	HLA tissue typing, paternity testing, DNA studies	8-10
8	Light Grey Top Glucose tube	Sodium fluoride and potassium oxalate	Antiglycolytic agent preserves glucose	Glucoses, requires full draw (may cause haemolysis if short draw)	8-10
9	Black Top ESR Tube	3.8% Sodium Citrate	Calcium salt formation	1:4 ratio between anticoagulant and blood	8-10

Note: Mix the samples containing additive by inverting the container as many times as times as specified. DO NOT SHAKE THE TUBE.

- **Biomedical Waste management; Sharps**
  - Burn and cut the needle and syringe using the needle destroyer. Perform the following steps:
  - Insert the needle in the needle slot
  - Push it gently downwards till arcing stops
  - Put the syringe in syringe slot.
  - Push the handle of the cutting blade to cut the syringe at the nozzle
  - The 'segregation at source' of biomedical waste is to be done as per local guidelines abiding to BMW 2016 rules (under Ministry of Environment and Forest)
  - Recapping the needle is not encouraged. Where recapping of a needle is unavoidable, use the one-hand scoop technique as described below:
  - Leave the needle cap on the surface and guide the tip of the used needle tip into it using only one hand.
  - Place the needle cap against a firm upright surface with its opening towards the phlebotomist, and place the used needle tip into it.
  - Lift the needle and syringe vertically and, once the tip is covered, use the other hand to fix the cap into place. Clean the surface with disinfectant afterward to avoid leaving any drop of blood.

NEVER DISASSEMBLE AN EXPOSED, USED NEEDLE WITH YOUR BARE HANDS

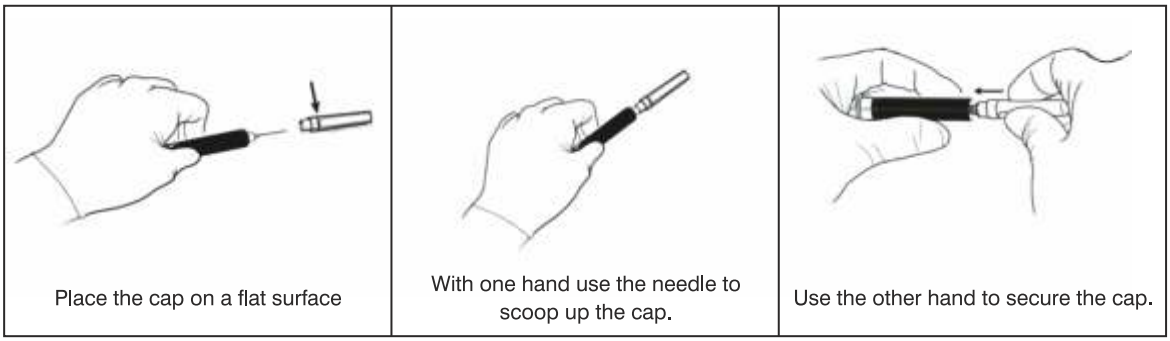


Figure 10: One hand scoop technique

- **Dismissing the patient:**
  - Check puncture site to ascertain if bleeding has completely stopped.
  - Apply sterile Band Aid on the puncture site or cotton swab: Optional
  - Dismiss the patient in a courteous and professional manner.

- **Verification of sample, logging of any incident:**

  Recheck the label on the sample tube and the request form before attending to the next patient
  - Any blood spill should be carefully managed as per procedure 'Management of Blood Spills' in Laboratory Safety Manual. **The events should be logged.** (See Annexure A)
  - For patients with disability or in critical condition, laboratory may make provision for bedside sample collection or in the room provided on ground floor.

- A log book should be maintained by technicians indicating the number of needle-pricks per patient to measure the pre-examination quality indicator for measuring the quality in primary sample collection procedure.

4.2.3 Special Collection Techniques: Venous

4.2.3.1 Winged Blood Collection for accessing fragile veins

- **Equipment**

  While accessing small / fragile veins (pediatric / geriatric patients) use of winged blood collection sets with 22/23 G needles recommended due to ease in manipulation to reduce stress exerted on vein (venous collapse), by modulating application of vacuum. A winged infusion set—also known as "butterfly" or "scalp vein" set—is a device specialized for venipuncture: i.e for accessing a superficial vein for either intravenous injection or phlebotomy. It consists, from front to rear, of a hypodermic needle, two bilateral flexible "wings", flexible small-bore transparent tubing (often 20–35 cm long), and lastly a connector (either female Luer or a Luer adapter that can fit on a tube holder). This connector attaches to another device: Such as syringe or Vacuum tube holder or hub. While using this device, the user must ensure that the connection is tight, otherwise there could be leakage of vacuum from a loose connection leading chances of froth formation and hence hemolysis.

Figure 11: Winged Collection Set

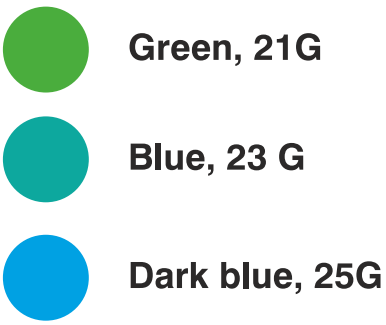


Figure 12: Color Codes for needle gauge

- **Needle Sizes and Color Coding**

  The winged blood collection sets are available in different gauges. As explained earlier, higher the G number – smaller is the size of the needle. The gauge number selected for a patient is related to the condition / size of the selected vein for venipuncture.

- **Holders**

In case of evacuated collections, it is recommended to use wing set and holders from the same manufacturer to ensure compatibility. Holders are clear and are molded to a standard shape. Wings or extensions at the sides of the tube end of the holder act as a lever against which the fingers or thumb can exert pressure to pop on or remove the evacuated tubes, while continuing to hold the needle steady within the patient's vein.

Figure 13: Holder

The reduced risk of accidental needle-stick injury by protecting the blood collector from the non-patient needle is a major advantage of using holders. Disposable holders with needle attached are disposed of as one unit, reducing the risk of accidental needle stick injury. Do not attempt to remove the needle and reuse a disposable holder, the threads on the holder are only designed to be used once and may cause the needle to release during collection. Larger sharps containers may be required to accommodate disposable holders

- **Assembling Winged Blood Collection Set**

When using wing set for evacuated closed collection system, holder is attached to the Luer adapter of the blood collection set. While using wing set with Luer adapter, a holder is attached to the wing-set. This would allow the phlebotomists to perform closed blood collection using evacuated tubes.


Figure 14: Assembled Winged Set

- **Winged Blood Collection Set with Safety Shield**

The wing set has a safety shield attached to the needle. After use, the patient end of the needle is easily and completely retracted into shield and locked in place. Hence helps in reducing chances of needle stick injury after use Majority of needle stick injuries happen within few seconds of the device usage. The activation of safety mechanism immediately after usage would help in reducing such needle stick injuries.

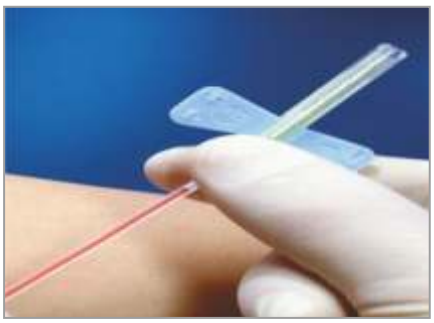


Figure 15: Winged set with safety shield

- **Site Selection in Hand**

In case the veins are not visible, one could consider wrapping a warm, wet towel around the hand for a few minutes. Warming the site increases the blood flow and helps make the veins easier to feel. Where there is uncertainty about a vein, tapping the site sharply a few times may assist.This helps dilate the vein and makes

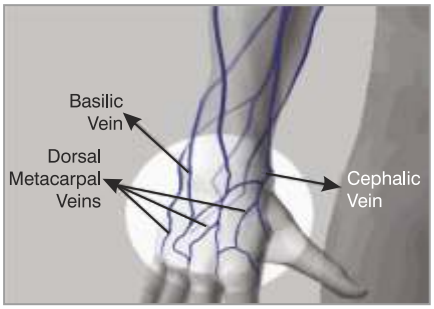


Figure 16: Veins on the Dorsum of Hand

it more prominent. The wrist veins tend to move or roll aside as the needle is inserted; therefore it is critical to hold the hand such that veins are well anchored. It is preferable to use a blood collection set to perform venipuncture on wrist veins.

- **Site Selection On Foot**

The blood collection from the foot veins should be a last resort, after the arm veins have been determined unsuitable. Institution policies must be followed. Blood flow in extremities (such as foot) may not be representative of general circulation (particularly in patients with vascular disease – e.g. diabetes) and may yield erroneous results. Further, complications in patients with diabetes, coagulopathies etc. can result in gangrene and thrombosis. Great care should be taken and only senior, experienced Phlebotomist / HCWs should attempt this procedure.

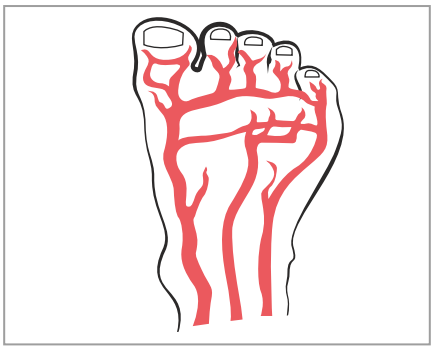


Figure 17: Veins on the Dorsum of Foot

- **Procedure**

Venipuncture Procedure using Winged Blood Collection Set:

- Grasp both wings of the blood collection set using index finger and thumb of dominant hand
- Hold the winged blood collection set as shown in the picture with holder or syringe attached to the non-patient end.
- While anchoring the veins and keeping skin taut with thumb of non-dominant hand, enter the vein at 10 - 15°angle
- If using evacuated collection push the tube into the holder using thumb while index and middle fingers grasp wings of the tube holder. If using syringe, slowly withdraw the syringe plunger
- Blood now begins to flow. Release the tourniquet

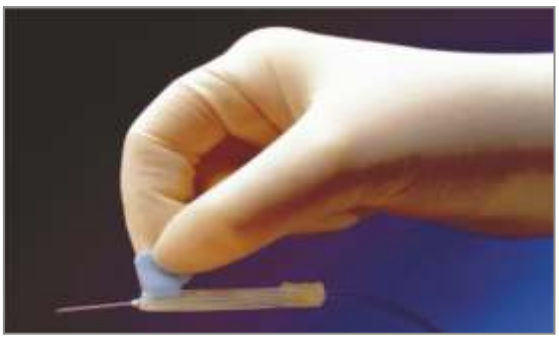


Figure 18: Angle of insertion of needle

Figure 19: Anchoring of vein by keeping the skin taut

Note: While using evacuated collection method follow the same order of draw as discussed earlier. Using a discard tube to avoid the risk of under-filling is recommended as the vacuum will pull in the dead space air in the butterfly needle's tubing before the blood gets drawn. Since plain tubes are not available in our country, a citrate tube can be used instead as a discard tube. This can be removed and discarded as soon as the blood enters it by which time the dead space is overcome

- **Withdraw the Needle**

  Once last tube has filled and been withdrawn or required amount of blood has been withdrawn in syringe, put a clean gauze pad on site and apply light pressure using three fingers as shown in the picture. Gently and quickly withdraw the needle and continue applying pressure on site

- **Activating Safety Feature**

  Withdraw the needle while grasping the safety shield area with thumb and index finger. With opposite hand grasp tubing between thumb and index finger. Push the safety shield forward until the shield locks in place. Discard the complete assembly into an approved sharp container.

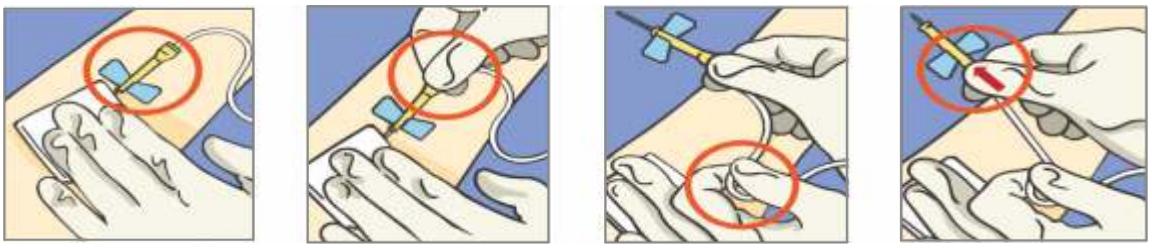


Figure 20: Activating the safety shield

- **Discard the Blood Collection Set**

  - Discard the complete assembly without removing the holder into an approved safety box
  - Be aware tubing attached to sharps can recoil and lead to injury – maintain control of both tubing and the device during disposal

Figure 21: Sharp disposal

4.2.3.2 Blood Cultures

A. Venous Blood

Requirements:

- Blood Culture bottle containing liquid broth medium, , povidone-iodine or equivalent iodine-containing agent, 70% Isopropyl Alcohol, a combination of 2% Chlorhexidine with 70% isopropyl alcohol

Indications

- Routine blood cultures should be performed on any patient in whom there is a suspicion of bacteremia or Candidemia. Surveillance for infection before the clinical suspicion of infection exists is not an indication for blood culture.

Timing and recommended procedure

- Blood cultures should be drawn prior to the institution of antibiotics whenever possible.
- If empiric treatment is an emergency, blood cultures should still be drawn as soon as possible after institution of antibiotics.
- Acute febrile episode: 2 sets from separate sites, all within 10 min (before antimicrobials)
- Non-acute disease: antimicrobials will not be started or changed immediately: 2 or 3 sets from separate sites, all within 24 hrs, intervals no closer than 3 Hrs (before antimicrobials)
- Acute endocarditis: 3 sets from 3 separate sites within 1-2 h, before antimicrobials if possible
- PUO: 2 or 3 set from separate sites, $\geqslant$1 h apart during 24 h period. If negative at 24-48 h, obtain 2 or 3 more sets

Site of Blood Culture

- Blood should be obtained from peripheral venous sites.

Preparation of the Site for Culture

- After the vessel site is selected, a 5 cm area of skin should be disinfected by swabbing concentrically with 70% isopropyl from the venipuncture site outward. Never touch inside again.
- The site should be cleansed once again, this time with 10% povidone-iodine (Betadine) again from center to periphery not touching the center again. Iodophors require 1.5 to 2 minutes contact time, while iodine tincture needs 30 seconds for its effect
- In patients allergic to iodine, a combination of 2% chlorhexidine with 70% isopropyl alcohol can be used
- Allow the iodine to dry completely before performing venipuncture. This takes about 1 - 2 minutes.
- While waiting for the site to dry, the plastic cap covering each blood culture bottle should be removed, and the rubber stopper should be decontaminated with 70% alcohol. (Iodine solutions will disintegrate the rubber and should not be used.)
- 7-10 of blood should be withdrawn from the puncture site.
- Use of winged blood collection set (explained above) with holder is preferred for collection of blood for blood culture. Most of commercial blood culture bottles have vacuum and hence blood would automatically flow in to the bottle under vacuum. After appropriate volume of blood has been collected in blood culture bottles, other samples can be collected following the order of draw indicated in previous section.
- While using syringe and needle to collect blood, do not change needles between venipuncture and inoculation of the bottles, or between bottles. The risk of needle stick is increased, while the chance of contamination is not significantly lessened.

- Remove the iodine solution from the skin with alcohol. This will minimize the possibility of hypersensitivity.
- Transportation time and temperature should be ⩽ 2 h, at Room Temperature; the storage temperature should be ⩽ 2 h, at Room Temperature or per instructions. Replica limits is 3 sets within 24 hrs.

- **Adults:**
  There is a direct relationship between the volume of blood and an increased probability of isolation of a pathogen. Therefore, collection of two sets of cultures using 10-20 mL of blood per culture is strongly recommended for adults. In addition to the volume of blood collected and type of medium chosen, the dilution factor for blood in the medium must be considered. Traditionally, a 1:10 ratio of blood to medium was required for successful bacterial growth; however, several new commercial media containing resins or other additives have demonstrated enhanced recovery of pathogens with as low as 1:5 ratio. All commercial media specify the volume and dilution.

- **Infants and Children:**

Table 4.3: Blood Culture Volume Requirements in Infants & Children

Weight of patient		Total blood volume (mL)	Recommended volume of blood for culture (mL)			% of Total blood volume
Kg	Lb		Culture no.1	Culture no. 2	Total volume of culture(mL)	
⩽1	⩽ 2.2	50-99	2	-	2	4
1.1-2	2.2-4.4	100-200	2	2	4	4
2.1-12.7	4.5-27	>200	4	2	6	3
12.8-36.3	28-80	>800	10	10	20	2.5
>36.3	>80	>2200	20-30	20-30	40-60	1.8-2.7

B. Intravenous Catheters

Intravenous (IV) catheters are an integral part of care for many hospitalized patients. Central venous catheters are used to administer fluids, blood products, medications, antibiotics and nutrition, and for hemodynamic monitoring. Unfortunately, a major consequence of these medical devices is colonization of the catheter by either bacteria or fungi, which can lead to catheter infection and serious bloodstream infection. This consequence is a major nosocomial source of illness and even death.

Common Agents of IV Catheter-Associated Bacteremia:

1. *Staphylococcus epidermidis*
2. *Other coagulase-negative staphylococci*
3. *Staphylococcus aureus*
4. *Enterobacteriaceae*
5. *Pseudomonas aeruginosa*
6. *Candida spp.*
7. *Corynebacterium spp.*
8. *Other gram-negative rods*

- **Techniques to detect IV Catheter-Associated Infections:**

  A. Semi-quantitative cultures B. Gram stains of skin entry site

  C. Culture of IV catheter tips

- **Equipment**
  - Sterile screw-cap tube or cup

- **Acceptable intravenous catheters for semi-quantitative culture:**

  Central line, CVP, Hickman, Broviac, peripheral, arterial, umbilical, hyperalimentation, Swan-Ganz

- **Procedure**
  - Cleanse the skin around the catheter site with alcohol.
  - Aseptically remove catheter and clip 5 cm of distal tip directly into a sterile tube. Some elect to culture the 5-cm intracutaneous portion to evaluate for soft tissue infection.
  - Transport immediately to microbiology laboratory to prevent drying.
  - The terminal end of IV catheter is removed and rolled several times across blood agar plate. The tip is removed from the agar plate and placed in enrichment broth. Both the plate and enrichment broth are incubated at 37°C for 18 to 24 hours. Following incubation, the blood agar plates are examined, and any isolate is identified according to laboratory protocol. The enrichment broth may be subcultured to blood agar and anaerobic media for further analysis.

4.2.4 Complications of Venipuncture

Table 4.4: Complications of venipuncture

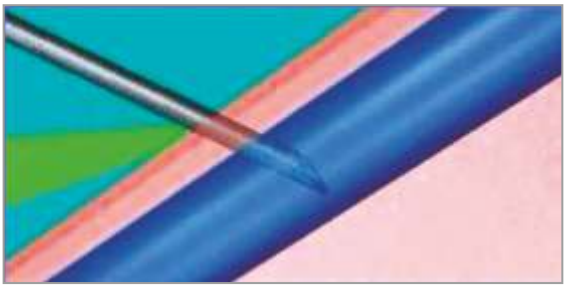
A) Local Complications	B) General	C) Sample Complication
Improper positioning of needle	Nausea	Hemolysis
Rolling of vein	Vomiting	Clots in anti-coagulated specimens
Puncture through vein	Fainting attack	Platelet clumps
Needle bevel obstruction	Convulsions / seizures	
Collapsed vein	Inadvertent arterial puncture	
Partially inserted needle	Nerve damage	
Tube pop-off		
Anticoagulant reflux		
Tourniquet not removed		
Excessive bleeding		
Petechiae		

A. Local Complications:

Improper Positioning of needle

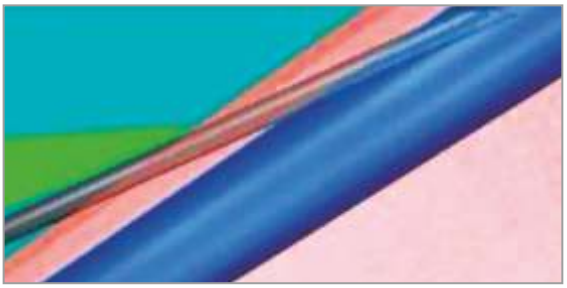
Bevel of needle fully inserted at 15-30 degree angle

Figure 22: Correct Position



Bevel of needle too steep. Potential for needle to completely penetrate the vein possibly resulting in formation of hematoma

Figure 23: Wrong Positions (Needle too steep)



Angle too shallow, bevel ocluded by wall or partially in vein/partially in ttissue. Possibility of developing haematom

Figure 24: Wrong Positions (Needle too shallow)

Rolling of the Vein

When a vein is not well-anchored prior to puncture, it may change position (roll) after or during the process of needle insertion. When a vein rolls, the needle may slip to the side of the vein without penetrating it. Reposition the needle and check for blood flow. If there is still no blood flow, remove tourniquet, ensure patient's hand is open, withdraw tube, and remove needle from patient's arm. Consider an alternative site on the opposite arm.

Puncture Through Vein

Sometimes the needle penetrates through both walls of the vein when bevel is inserted at steep angle

Needle Bevel Obstruction

Occasionally the bevel of the needle when the angle is too low and the needle lies against the wall of the vein preventing free blood flow. Pull back slightly on the needle. If there is still no blood flow, remove tourniquet, ensure patient's hand is open, withdraw tube, and remove needle from patient's arm. Avoid rotating needle more than 1/4th turn as it may damage vessel wall.

Collapsed Vein

Sometimes vacuum draw of tube, or pressure created by pulling the plunger of a syringe, causes a vein to collapse. Blood flow slows and then stops as vein collapses.

Collapse early on may be due to insufficient tourniquet pressure. .After about 5 ml this may be due to excessive tourniquet pressure. This may also occur when tourniquet is applied too close to site.

Experiment with tourniquet pressure (increase or decrease as appropriate).If still no blood flow, remove tourniquet, ensure patient's hand is open, withdraw tube, and withdraw needle

Partially Inserted Needle - Hematoma:

If bevel of needle is not totally within lumen of vein, blood will leak into surrounding tissue causing a hematoma and reduced blood flow into tube

Release tourniquet and withdraw needle immediately. Apply firm pressure to site for several minutes. Ask patient to maintain pressure for prolonged period (if possible) or request assistance from nursing staff as appropriate

Tube 'Pop Off' (for evacuated tubes only):

Occasionally, during a venipuncture, a needle sleeve may push the tube off the needle slightly, as a result blood flow stops. Pop-off' can be due to excessive lubricant on the non-patient end of the needle (manufacturing fault) or a faulty MSN sleeve (also a manufacturing issue). May also be operator induced (insufficient force applied to the tube to ensure it is completely inserted onto the non-patient needle.

To re-establish blood flow, re-advance the tube to the end of the holder and maintain it in this position until the tube is filled.

Reflux of Anticoagulant (for evacuated tubes only):

If tube is not properly oriented and tourniquet is suddenly released, pressure inside tube may momentarily exceed that in the vein. Blood might then flow back into patient's vein (reflux) from the collection tube Tube additives, (eg EDTA) have the potential to cause adverse reactions in patients. To prevent reflux, the patient's arm should be maintained in a downward position to ensure the collection tube remains below the venipuncture site and fills from the bottom upwards. Prolonged tourniquet application increases the risk of reflux. Reflux is of greatest concern when non-sterile tubes are used because of the potential of transferring harmful micro-organisms to the patient's peripheral blood (and consequent bacterimia septicaemia) involving bacterial (and other micro-organism) contamination is much more of a concern than reflux of additives as the latter will be greatly diluted in the blood.

Tourniquet Not Removed:

Failure to remove tourniquet before withdrawing needle maintains pressure inside vein. Blood can spill out of vessel once needle is removed creating Biohazard risk, Patient anxiety, Hematoma. Tourniquet tension should be reduced (or the tourniquet removed) as soon as blood starts flowing

Inadvertent Arterial Puncture:

Arterial blood has a bright red color and the tube fills very quickly,in such a case remove needle and hold pressure for at least 5 minutes

Excessive Bleeding:
If bleeding happens for longer than 5 minutes alert nurse and notify attending physician, continue pressure on site as long as necessary to stop bleeding, wrap bandage securely around arm over gauze pad, leave it on the site for at least 15 minutes.

Petechiae:
Red spots under the skin, maybe due to tourniquet left on arm or may represent excessive capillary fragility in some patients. Inform the attending physician and-avoid / limit use of tourniquet application

Nerve Damage:
Avoid major nerves. Contact with nerve tissue with a needle can cause sharp and immediate pain and may also induce an involuntary reflex action such as pulling the arm away from the needle. Both the median nerve and the brachial artery lie close to the basilic vein. Excessive or blind probing while performing a venipuncture can lead to permanent injury of the nerve or artery that may result in legal action.

B. General Complications:

Nausea: Make patient as comfortable as possible and Instruct patient to breathe deeply and slowly. Apply cold compresses on patient's forehead. Notify personnel trained in first aid

Vomiting: Same steps as in Nausea and give patient water to rinse out mouth

Fainting (Vasovagal syncope):
Notify personnel trained in first aid, lay patient flat or lower his/her head and arms. If sitting, loosen tight clothing and cease procedure. One of the main concerns in such situations is to ensure patient safety against fall / injuries. The need for usage of phlebotomy chairs with arm rests and dissuading specimen collection while the patient is standing should be reemphasized. Such events also increase risk of NSI due to unexpected movement of patient and panic response. Needle should be removed carefully.

Important to seek help from another HCW. It is better to make a note of such an occurrence on the requisition form.

Figure 25: Vasovagal Attack, Management

Convulsions / Seizures: Call for help and cease procedure. Have someone hold pressure to site and notify personnel trained in first aid. Lower patient to floor and clear space to prevent injury and do not restrain patient's extremities

C. <u>Specimen Complications:</u>
Specimen Quality Compromise like Hemolysis, clot in anticoagulated sample and Platelet clumps can happen due to improper collections

<u>Hemolysis</u>
Rupture of red blood releasing of its constituents: Affects several analytes: RBC, HCT, K+, LDH, AST, ALT, Iron, Phosphorus, Proteins, Magnesium,Calcium etc.

Table 4.5 Causes of hemolysis

Possible factor affecting hemolysis	Possible consequences
Vein	Fragile and easily traumatized veins
Prolonged tourniquet application	Hemoconcentration, RBC rupture
Venipuncture site cleansing procedure	Performing venipuncture before alcohol is thoroughly dry
Needle Readjustment	Vein trauma
Needle Gauge Lumen too large Lumen too small, smaller than 23	Blood enters tube faster Blood enter under high vacuum
Syringe Collections	Too much force applied to plunger, thus increasing the shear force on the red blood cells;
Forceful transfer of blood into tubes	Increasing the shear force on the red blood cells
Mixing a tube too vigorously;	Increasing the shear force on the red blood cells
Using too great a vacuum; for example, using too large a tube for a pediatric patient, or using too large a syringe (10–20 ml).	Increasing the shear force on the red blood cells
Under filling a tube so that the ratio of anticoagulant to blood is greater than 1:9	
Reusing tubes that have been refilled by hand with inappropriate amounts of anticoagulants	
Abruptly stopping a draw (e.g. withdrawing a 6 mL tube when half of it is filled)	

Clot in Anti coagulated Sample

Impact: Specimen rejection in the lab, instrument downtime due to probe clogging and error in test results

Causes: Overfilling of tubes, inadequate mixing, difficult draw, low filling of evacuated tubes

Platelet Clumping

Impact: Reduced platelet count, Increased RBC count

Cause: Poor mixing and over filling additive tubes

4.3 Capillary Blood Collection

4.3.1 Indications in Adult Patients:

- Severe burns
- Extreme obesity
- Hypercoagulability (thrombotic tendency)
- Geriatric or fragile veins
- Need to preserve veins for therapy
- Home testing
- Apprehensive patients
- Point of care testing

4.3.2 Tests Commonly Performed on Capillary Blood:

- Blood Smear
  - o Microscopic assessment of cell morphology
  - o Preparation for detection of malarial parasites
  - o Estimation of platelet count
  - o WBC morphology
- Rapid tests e.g. HIV antibody detection
- Dried blood spot for early infant diagnosis of HIV infection
- Blood glucose monitoring
- Pre-blood donation testing
- Biochemistry tests available in the capillary mode analyzers. e.g. Bilirubin, Thyroid functions
- Hematology tests available in the capillary mode analyzers e.g. CBC
- Bleeding time Ivy's method

Note: Blood culture cannot be performed on capillary blood

4.3.3 Equipment and supplies:

- Lancets
- Specimen collection equipment
- Filter Paper (Dry blood spot or DBS)
- Capillary tube
- Test specific equipment
- Blood smear slides
- Rapid test devices
- Micro-collection tube
- Filter Paper

- **Lancets**

  - **Manual Lancets**

Single use lancets are available but the depth of penetration with these cannot be controlled. The Lancet could pose risk of sharps injury to healthcare worker after use.

  - **Safety Lancets; separate lancet available for finger and heel puncture**

Single use lancets for different depths available which activates only when it is positioned and pressed against the skin. The depth will never exceed the specified depth for the device, facilitating a consistent puncture depth for easier sampling.

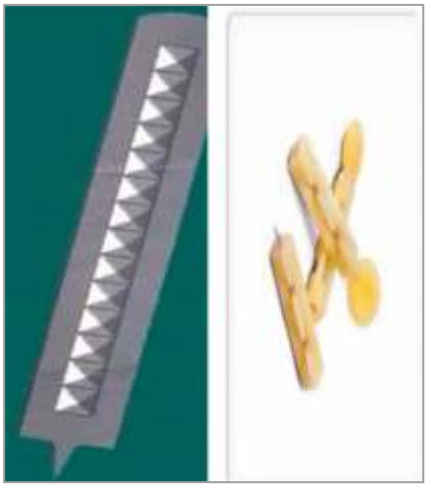


Figure 26: A & B manual lancets

Puncture depths

- < 2.0 mm for all heelstick
- < 1.5 mm for fingerstick on children over 12 months
- < 2.0 mm for fingerstick on children over 8 yrs
- < 2.4 mm for fingerstick on adults

Techniques

- Blood Smear on Slide: Conventional Method
- Rapid Tests as specified by the manufacturer
- Capillary Methods For different Tests e.g. Serum Bilirubin. Instructions of the manufacturer are to be followed. Glass or plastic clad capillaries are available, with or without anticoagulant. Plastic clad capillaries are safer as they are non-sharp. Collect blood directly into a capillary tube. After required amount of blood has been collected, seal the tube using plastic, clay material. Label individual capillary by wrapping label around it like a flag

- **Micro-collection Tubes**
  - Micro-collection tubes are non evacuted and designed for collection, transport and processing of capillary blood. They are color coded similar to evacuated tubes

- **Dried Blood Spot (DBS)**
  - These are blood spots on special filter papers. DBS offers ease of specimen collection and transportation through post. This is utilized for variety of tests like, Early infant diagnosis (EID), HIV viral load (under research) and IBBS - HIV sentinel surveillance.

4.3.4 Procedure:

- Selecting Equipment and Supplies
- Verify information on test requisition form and select micro-collection devices
- Assemble the supplies and choose the appropriate puncture site

Site Selection for Capillary Collection:

Table No: 4.6: Conditions influencing the choice of heel or finger-prick

Condition	Heel-prick	Finger-prick
Age	Birth to about 6 months	Over 6 months
Weight	From 3 -10 kg, approximately	Greater than 10 kg
Placement of lancet	On the medial or lateral plantar surface	On the side of the ball of the finger perpendicular to the lines of the fingerprint
Recommended finger	Not applicable	Second and third finger (i.e. middle and ring finger); avoid the thumb and index finger because of calluses, and avoid the little finger because the tissue is thin

Heel

Neonates and Infants up to ≈ upto 6 months depending on weight (See table)

Use plantar surface of the heel (medial to a line drawn posteriorly from the large toe to the heel or lateral to a line drawn posteriorly from between the 4th and 5th toes to the heel)

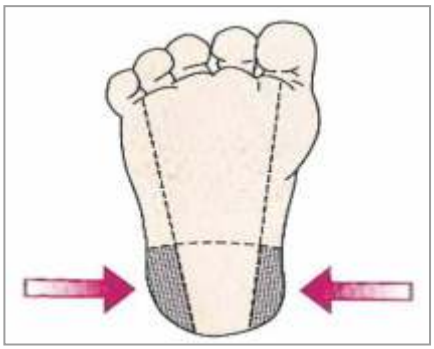


Figure 27: Site selection Heel

Site Selection for Capillary Collection - Finger

Infants >6 mth-1 year, older children and adults Use palmar surface of tip of 3rd or 4th finger

Steps

- Warming

If possible, gently warm the puncture site as warming can increase blood flow by 7-fold. A warm, moist towel OR warming device may be used for this which should be heated higher than 42o and a contact time 3 to 5 minutes is to be given

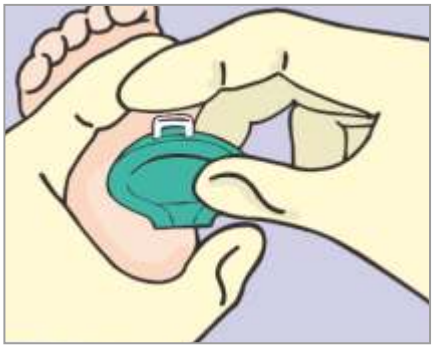


Figure 28: Heel puncture with the safety device

- Site Cleansing

All precautions as mentioned in the venous collection to be followed. Do not use Betadine / povidine iodine (↑ K+, PO4-, UA)

- Skin Puncture – Finger

Inform grown up patient about imminent pain. Hold the finger and firmly place a new sterile lancet at the selected site on the finger. If using self-retracting safety lancet, activate the lancet / If using manual lancet perform single puncture with one smooth motion

- Skin Puncture - Heel

Hold the foot with a firm grip. Grasp heel with thumb placed below puncture site and index finger placed over the arch. Hold and orient the lancet on the intended puncture site. Firmly and completely depress the trigger of the lancet (safety lancet) or perform single puncture with smooth motion using manual lancet. Puncture site at a 90 degree angle to the length of the foot

Heel Puncture Precautions

- Do not puncture deeper than 1.5 mm (unless instructed otherwise)
- Do not puncture through previous punctures
- Do not puncture outside the medial and lateral aspects of the heel previously described
- Do not puncture the posterior curvature of heel
- Do not puncture in the arch
- Do not puncture areas of the foot other than the heel

- Must discard first drop of blood to avoid specimen dilution by tissue fluid .The excess of tissue fluid would dilute the sample and reduce the concentration of all the analytes thus affecting the results.Never milk or scrape the puncture site. Collect the sample beginning with the second drop using any of the collection devices:
- Blood flow is enhanced by holding the puncture site downward and gently applying intermittent pressure to the surrounding tissue. Continue until desired volume has been collected Follow recommended order of draw for micro collection tubes. While collecting blood in micro collection tubes collect EDTA tube before serum tube Mix anticoagulant containing micro collection tubes continuously during collection
- When blood collection is complete, apply pressure with a clean, dry gauze pad until bleeding stops. Label specimens immediately after the draw
- Do not use adhesive bandages on children less than two years old as it may irritate infant's skin
- Ensure proper disposal of the lancets and other consumables as per institutional guidelines

Dried Blood Spot, Preparation and Transportation

One of the major applications of capillary collection is in Dried Blood Spots. The following steps may be observed while collecting DBS samples

- Ensure the spots fill in the circles printed on the filter paper and are not over saturated
- Ensure sufficient blood on each spot
- Ensure that there are no scratches on spots
- Ensure no scattered spots
- Ensure no more than one layer
- Ensure the blood spots are completely dry
- Label the glycine storage bags
- Insert each filter paper into appropriately labeled bag and add small desiccant
- Seal the bag
- Insert sealed bag into a zip-lock bag (5 in 1)
- Add one packet of desiccant
- Add one humidity indicator card
- Seal the zip-lock bag immediately
- Avoid touching or smearing the blood spots
- Allow the specimen to fully air dry horizontally over night at room temperature
- Keep away from direct sunlight and protect from dust
- Do not heat, stack or allow DBS to touch other surfaces during the drying process
- Completely dry blood spots before packaging

- Label the glycine storage bags
- Insert each filter paper into appropriately labeled bag and add small desiccant
- Seal the bag
- Insert bundled DBS into rip-resistant envelope
- Include appropriate documentation
- Insert both into brown envelope and seal for transportation

4.3.5 Complications of Capillary Blood Collection:

- Collapse of veins if the tibial artery is lacerated from puncturing the medial aspect of the heel.
- Osteomyelitis of the heel bone (calcaneus). Contact with the heel bone (calcaneus) may cause osteomyelitis. Osteomyelitis could go undetected for long periods of time and could result in serious complications for young children.
- Nerve damage if the fingers of neonates are punctured
- Hematoma and loss of access to the venous branch used
- Scarring
- Localized or generalized necrosis (a long-term effect);
- Skin breakdown from repeated use of adhesive strips (particularly in very young or very elderly patients) – this can be avoided if sufficient pressure is applied and the puncture site is observed after the procedure.

4.4. Arterial Blood Collection

4.4.1 Background information on arterial blood collection

An arterial blood sample is collected from an artery, primarily to determine arterial blood gases.

Arterial blood sampling should only be performed by health workers who are legally authorized to do so and who have demonstrated proficiency for the procedure after formal training.

The sample can be obtained either through a catheter placed in an artery, or by using a needle and syringe to puncture an artery. These syringes are pre-heparinized and handled to minimize air exposure that will alter the blood gas values. This chapter describes only the procedure for a radial artery blood draw.

4.4.2 Choice of Site

Several different arteries can be used for blood collection. The first choice is the radial artery, which is located on the thumb side of the wrist; because of its small size, use of this artery requires extensive skill in arterial blood sampling. Alternative sites for access are brachial or femoral arteries, but these have several disadvantages in that they:

- may be harder to locate, because they are less superficial than the radial artery;
- have poor collateral circulation;
- are surrounded by structures that could be damaged by faulty technique.

4.4.3 Equipment and Supplies

Assemble the relevant items, plus the following specimen collection equipment and supplies:

- Homemade syringes (Hypodermic syringes flushed with sodium heparin) or Pre-heparinized syringe;
- Needles (20, 23 and 25 gauge, of different lengths) – choose a size that is appropriate for the site (smaller gauges are more likely to lyse the specimen)
- A safety syringe with a needle cover that allows the needle to be capped before transport, without manually recapping (this is best practice for radial blood sampling);
- A bandage to cover the puncture site after collection;
- A container with crushed ice for transportation of the sample to the laboratory (if the analysis is not done at the point of care);
- Where applicable, local anesthetic and an additional single-use sterile syringe and needle.

4.4.4 Procedure for Arterial Blood Collection using Radial Artery

For sampling from the radial artery using a needle and syringe, follow the steps outlined below.

1. Approach the patient, introduce yourself and ask the patient to state their full name.
2. Place the patient on their back, lying flat. Ask the nurse for assistance if the patient's position needs to be altered to make them more comfortable. If the patient is clenching their fist, holding their breath or crying, this can change breathing and thus alter the test result.
3. Locate the radial artery by performing an Allen for collateral circulation (see below). If the initial test fails to locate the radial artery, repeat the test on the other hand. Once a site is identified, note anatomic landmarks to be able to find the site again. If it will be necessary to palpate the site again, put on sterile gloves.
4. Perform hand hygiene, clear off a bedside work area and prepare supplies. Put on an impervious gown or apron, and face protection, if exposure to blood is anticipated.
5. Disinfect the sampling site on the patient with 70% alcohol and allow it to dry.
6. If the needle and syringe are not preassembled, assemble the needle and heparinized syringe.
7. Holding the syringe and needle like a dart, use the index finger to locate the pulse again, inform the patient that the skin is about to be pierced then insert the needle at a 45 degree angle, approximately 1 cm distal to (i.e. away from) the index finger, to avoid contaminating the area where the needle enters the skin.
8. Advance the needle into the radial artery until a blood flashback appears, then allow the syringe to fill to the appropriate level. DO NOT pull back the syringe plunger.

9. Withdraw the needle and syringe; place a clean, dry piece of gauze or cotton wool over the site and have the patient or an assistant apply firm pressure for sufficient time to stop the bleeding. Check whether bleeding has stopped after 2–3 minutes. Five minutes or more may be needed for patients who have high blood pressure or a bleeding disorder, or are taking anticoagulants.
10. Activate the mechanisms of a safety needle to cover the needle before placing it in the ice cup. In the absence of a safety-engineered device, use a one-hand scoop technique (as explained in Annex G) to recap the needle after removal.
11. Expel air bubbles, cap the syringe and roll the specimen between the hands to gently mix it. Cap the syringe to prevent contact between the arterial blood sample and the air, and to prevent leaking during transport to the laboratory.
12. Label the sample syringe.
13. Dispose appropriately of all used material and personal protective equipment.
14. Remove gloves and wash hands thoroughly with soap and water, then dry using single use towels; alternatively, use alcohol rub solution.
15. Check the patient site for bleeding (if necessary, apply additional pressure) and thank the patient.
16. Transport the sample immediately to the laboratory, following laboratory handling procedures.

4.4.5 Complications Related To Arterial Blood Collection

Patient Complications

There are several potential complications related to arterial blood sampling. The points below list some of the complications related to the procedure, and how they can be prevented.

- Arteriospasm or involuntary contraction of the artery may be prevented simply by helping the patient relax; this can be achieved, for example, by explaining the procedure and positioning the person comfortably.
- Haematoma or excessive bleeding can be prevented by inserting the needle without puncturing the far side of the vessel and by applying pressure immediately after blood is drawn. Due to the higher pressure present in arteries, pressure should be applied for a longer time than when sampling from a vein, and should be supervised more closely, to check for cessation of bleeding.
- Nerve damage can be prevented by choosing an appropriate sampling site and avoiding redirection of the needle.
- Fainting or a vasovagal response can be prevented by ensuring that the patient is supine (lying down on their back) with feet elevated before beginning the blood draw. Patients requiring arterial blood sampling are usually inpatients or in the emergency ward, so will generally already be lying in a hospital bed. Children may feel a loss of control and fight more if placed in a supine position; in such cases, it may be preferable to have the child sitting on the parent's lap, so that the parent can gently restrain the child.

- Other problems can include a drop in blood pressure, complaints of feeling faint, sweating or pallor that may precede a loss of consciousness.

Sample errors

Inappropriate collection and handling of arterial blood specimens can produce incorrect results.

Reasons for an inaccurate blood result include:

- Presence of air in the sample;
- Collection of venous rather than arterial blood;
- An improper quantity of heparin in the syringe, or improper mixing after blood is drawn;
- A delay in specimen transportation.

Modified Allen test

A modified Allen test measures arterial competency, and should be performed before taking an arterial sample. The procedure for performing the test is as follows

- Instruct the patient to clench his or her fist; if the patient is unable to do this, close the person's hand tightly.
- Using your fingers, apply occlusive pressure to both the ulnar and radial arteries, to obstruct blood flow to the hand.
- While applying occlusive pressure to both arteries, have the patient relax his or her hand and check whether the palm and fingers have blanched. If this is not the case, you have not completely occluded the arteries with your fingers.
- Release the occlusive pressure on the ulnar artery only to determine whether the modified Allen test is positive or negative.
    - Positive modified Allen test – If the hand flushes within 5–15 seconds it indicates that the ulnar artery has good blood flow; this normal flushing of the hand is considered to be a positive test.
    - Negative modified Allen test – If the hand does not flush within 5–15 seconds, it indicates that ulnar circulation is inadequate or nonexistent; in this situation, the radial artery supplying arterial blood to that hand should not be punctured.

Chapter 5

Sample Collection: Specimen Other Than Blood

5.1 Learning Objectives

At the end of this chapter, the learners should be able to understand:

a) The equipment and concepts used in pre-analytical processes for specimen other than blood

b) The requirements for patient collected, lab collected and physician collected samples

c) The best practices to avoid errors

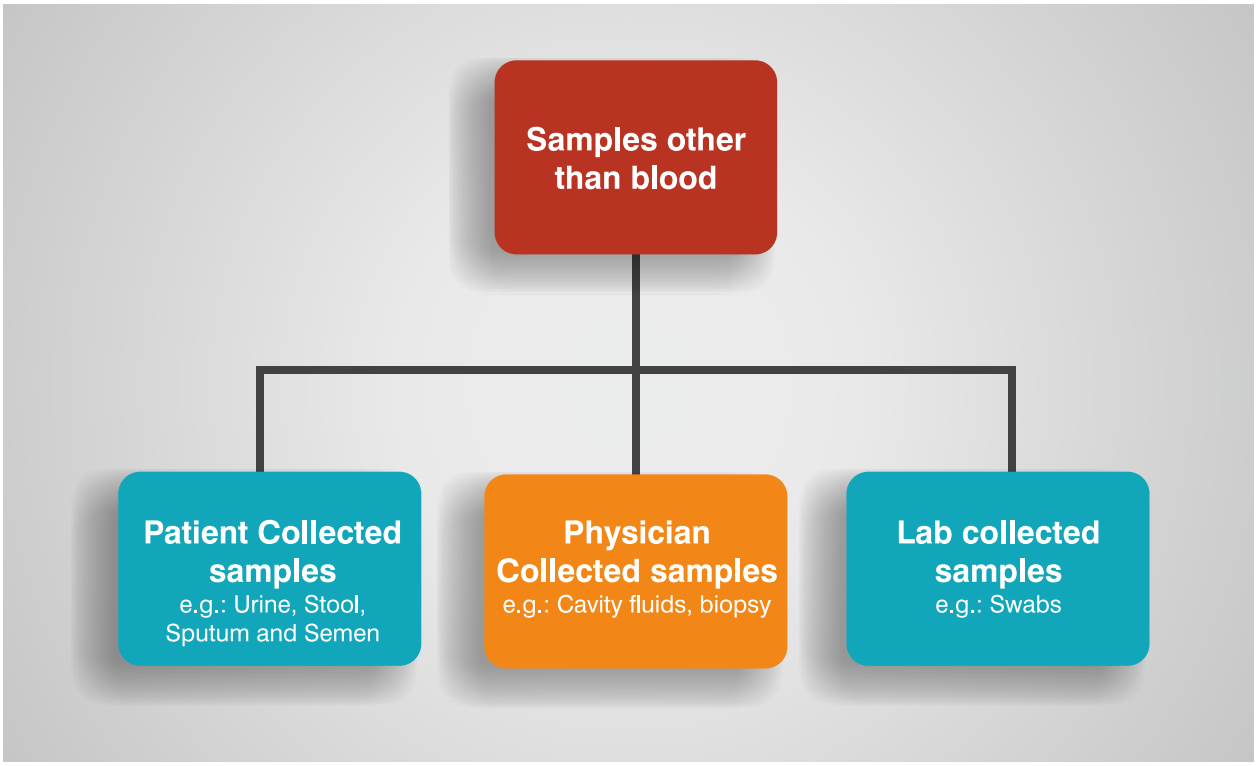


Figure: 29 Samples other than blood

5.2. Patient Collected Samples

5.2.1 Urine Collection

5.2.1.1 Spot Samples: Urine

- Routine Urine Examination,
- Urine Culture and Sensitivity and
- Other Spot Samples

For all urine analysis, early morning first voided clean catch mid-stream specimen is the best. If the patient presents with dysuria, spot sample may be accepted for routine as well as culture examination.

Requirements:

- Soap and water, Sterile Plastic Container
- Label container in legible, complete, and correct details (Patients Name, Age, ID number)

Method:

Clean Catch Midstream Urine:

It is the least invasive procedure and must be performed carefully for optimal results, especially females. Good patient education is essential. Guidelines for proper specimen collection should be prepared on a printed card (in local language, if necessary), with the procedure clearly described and preferably illustrated to ensure patient compliance. The

patient should be instructed to clean the periurethral area well with a mild detergent to avoid contamination. Of importance, the patient should also be instructed to rinse well because the detergent may be bacteriostatic. Once cleansing is completed, the patient should retract the labial folds or glans penis, begin to void, and then collect the midstream urine sample after discarding the initial flow.

Specimen Transport:

Because urine is an excellent supportive medium for growth of most bacteria, urine must be immediately refrigerated or preserved. Bacterial counts in refrigerated (4°C) urine remain constant for as long as 24 hours. Urine transport tubes containing boric acid, glycerol and sodium formate have been shown to preserve bacteria without refrigeration for 24 hours when greater than 10^5 CFU/mL (100,000 organisms per mL) were present in the initial urine specimen. The system may inhibit the growth of certain organisms and it must be used with a minimum of 3 mL of urine. Both boric acid products preserve bacterial viability in urine for 24 hours in the absence of antibiotics.

5.2.1.2 Timed Collection: 24 hours Urine

Table: 5.1 List of Preservatives
(The preservative will depend on the analytical specifications)

Test on urine specimen	Preservatives
Urinary Phosphorus	6 M Hcl
Urinary Uric Acid	5% w/v NaOH
Urinary Calcium	6 M Hcl
Urinary Magnesium	12 M Hcl
17 OH Corticosteroids	6 N Hcl
17 Ketosteroids	Conc. Hcl
Catecholamines	6 N Hcl
Metanephrines	6 N Hcl
Urinary Cortisol	Boric acid
5 Hydroxyindoleacetic acid	6 N Hcl
Vanilylmandelic Acid	6 N Hcl
Urinary Oxalate	Conc. Hcl

- All collection containers to be labeled with the identifiers like patient's name, age and number. Specific labels are to be attached to the container with instructions about collection.
- If specific preservatives are required for different analytes of biochemistry, instruct the patient about the preservative and restrict him from discarding the same.
- Instruct him/her to discard the first urine passed in the morning. Note down the exact time (e.g. 8.00am). From this time onwards collect all subsequent urine samples in the

container provided. Collection should be continued till same time next day (e.g. 8.00 AM). The 8 AM sample voided also should be collected.

- The patient should return the 24 hours urine sample container within 1 hr. to the lab.
- The details of time should be entered on the container by the patient.

Note: For Creatinine Clearance Test take one blood sample for Serum Creatinine during the 24 hours.

Microalbumin: Spot samples are adequate

Urine Pregnancy Test: Early morning clean catch midstream specimen is preferable.

5.2.1.3 Urine Collection from Catheterized Patients:

Requirements: Sterile containers, spirit swab, clamp, sterile syringe and needle

- Never collect sample from urine bag;
- Never accept the cut Foley's catheter specimen from patient;
- Clamp the catheter distally to allow urine to collect;
- Collect from the catheter tube after cleaning it with a spirit swab;
- Using a sterile syringe and needle collect sufficient amount of urine sample;
- Transfer to a sterile container;

Foley's Catheter for Culture

- Do NOT culture, since growth represents distal urethral flora and gives erroneous result
- Not acceptable for culture

5.2.2 Stool Collection

Requirements:

- Wide-mouth container preferably with a scoop/ sterile container/ swab

Collection Procedure:

5.2.2.1 Stool Routine

- Collect the stool sample in a clean, wide-mouthed screw capped pre-labelled container. For some stool tests (e.g. giardiasis), patients may be instructed to collect three consecutive stool samples on three days. Because organisms may be shed intermittently, collection of specimens at different times over several days enhances recovery.
- Do not collect the specimen from bed pan/closet.

- 1 to 2 gm quantity is sufficient.
- The fresh stool specimen must be delivered to the laboratory within 30 minutes of collection.
- Collect the sample in a clean wide-mouth container.
- Do not mix urine with the specimen.
- Tightly cap the container.
- Do not add any preservative in the sample.

5.2.2.2 Stool Occult Blood

The patient should be advised **not to collect** sample if:

- He /she has active bleeding from hemorrhoids or an anal fissure or when there is blood in urine. Women should be advised not to collect during menstrual period or during or the first three days after the end of the period.
- The patient should be advised to stop taking aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen seven days before faecal occult blood test.
- The patient should be advised to make dietary changes 3 days prior to faecal occult blood test.
- Avoid taking more than 250 milligrams of vitamin C per day. Avoid eating red meat (beef or lamb). Avoid eating raw fruits and vegetables, especially melons, radishes and turnips. These foods and supplements can produce a positive faecal occult blood test even when there is no bleeding from the digestive tract. This is called a false-positive test.
- Toilet-bowl cleaners may affect the results of the test so the sample should be collected with the least contact with the toilet –bowl.

5.2.2.3 Stool Culture

Procedure:

- Collect specimen (liquid - at least 5 ml in volume) directly into a clean, dry sterile, leak proof, wide mouthed container,
- Transport to microbiology laboratory within 1 h of collection or transfer to Cary-Blair or other appropriate transport medium.
- Stool for direct wet-mount examination, Clostridium difficile toxin assay, immunoelectron microscopy for detection of viruses, and ELISA or the latex agglutination test for rotavirus must be sent to the laboratory without any added preservatives or liquids.
- The Cary-Blair transport medium preserves the viability of intestinal bacterial pathogens including Campylobacter and Vibrio spp.
- For detection of ova and parasites, specimen preservation with a fixative is recommended for visual examination.
- Stool for virus culture must be refrigerated if they are not inoculated into cell cultures within 2 hours.

- Culture for routine pathogens is not recommended unless otherwise recommended by physician
- Other specimens that may be obtained include duodenal aspirates, string test, rectal swab.

Swabs for routine pathogens (parasites, toxins or viral antigens) are not recommended except in infants

5.2.3 Sputum Collection

Requirements:

- Sterile plastic screw capped container

Procedure

- Select a good wide-mouthed sterile sputum container, made of clear thin plastic, unbreakable and leak proof material. Label appropriately.

5.2.3.1 Expectorated

Explain to the patient to rinse his/her mouth with plain water before bringing up the sputum. It is essential that sputum, containing purulent material or destroyed tissue brought by a deep cough and not saliva, be collected. The patient is instructed to inhale deeply (2–3 times), which will initiate the cough reflex in most patients. The sputum is retained in the mouth and spit into the pre labeled container without spilling. Some patients may not be able to expectorate with deep breathing in which case you should demonstrate to them how they should place their palms on the waist, squat or sit and continue deep breathing again. (Sitting and placing hands on the waist fixes the shoulder and pelvic muscles and brings the inter-costal muscles of ribcage and diaphragm into action). Tapping or thumping of the back may encourage expectoration. Steam inhalation may help in the expectoration. Recover sample and place in a leak proof, sterile container. Whenever possible, sputum should be collected in an open place or in a well-ventilated room meant for this purpose. Sputum should not be collected in closed rooms, toilets and ill-ventilated rooms. The person collecting the specimen should make sure that no one stands in front of the patient who is trying to cough up sputum.

- Early morning sputum or **Spot Morning Spot (for TB diagnosis)** as advised by RNTCP may be followed.

5.2.3.2 Induced

Patients unable to produce sputum may be assisted by medical staff. Before sputum collection, patients should brush the buccal mucosa, tongue, and gums with a wet toothbrush. Alternatively, aerosol-induced sputum may be collected by allowing the patient to breathe aerosolized droplets, using an ultrasonic nebulizer containing 10% 0.85% NaCl or until a strong cough reflex is generated.

(Gastric aspirate: Used exclusively for isolation of acid-fast bacilli and may be used in children. Done by trained staff)

5.2.4 Semen Collection

Equipment

- Sterile, dry, wide mouth plastic container

Collection:

- Ensure a quiet separate room for semen collection.
- A period abstinence between 3 and 5 days is mandatory. Longer periods of abstinence usually result in higher semen volume but lower motility;
- If the period of abstinence is very long a second sample is to be collected after 2 hours to assess motility;
- Bladder should be evacuated prior to ejaculation;
- A post ejaculation urine sample also must be collected if retrograde ejaculation is suspected;
- The most satisfactory sample is when the collection is done in the lab to enable assessment of liquefaction time;
- If the sample is collected at home, then it should be delivered to the lab in 1 hour;
- Sample should not be collected in condoms as the powder or lubricant applied in condoms can be spermicidal;
- Home sterilised containers should not be used as detergents can be spermicidal;
- Water is spermicidal; the container must be dry;
- The lid of the container should not have rubber lining as contact with rubber can result in sperm death;
- The samples should be examined as early as possible. If delay is likely, store at 20˚C- 30˚C. Excessive, heat, cold and direct sunlight should be avoided. Do not store in refrigerator.
- Maintain confidentiality.

5.3 Lab Collected/ Physician Collected: Microbiology Sample Collection

Note: Blood culture has been explained along with the venous sample collection procedures. Urine and stool cultures have been explained in the appropriate sections. The outlines of other samples are explained below.

5.3.1 General Instructions

- Swabs from the genital tract, throat, eye, ear, nose, and superficial wounds (e.g., sores, boils, and rashes) should be transported to the laboratory preferably in the transport medium provided by laboratory (e.g. charcoal transport medium).
- **The patient's full name, date of birth or Unique Identification Number, the anatomical site swabbed and date and time of collection should be specified on the test requisition form and specimen container** to assist in the evaluation of bacteria isolated. Also, include any information concerning pregnancy or antibiotic drug allergies, therapy and other relevant clinical information on the test requisition form.
- All swabs have expiry dates, so rotation of stock is important. Dry swabs received for culture should **not** be processed unless received within one hour of collection.

5.3.2 Specimen Safety Considerations

- Follow standard precautions. Treat all specimens as potentially infectious.
- Laboratory workers should use appropriate PPE (such as gloves, laboratory coat or gown) when collecting or handling specimens. If splashing is likely to occur protective eyewear, face masks, and aprons may be worn.
- Do not contaminate the external surface of the collection container and/or accompanying form.
- Minimize direct handling of specimens in transit from the patient to the laboratory.
- Use plastic sealable bags with a separate pouch for the laboratory requisition orders or transport carriers (for example, small buckets with rigid handles).
- If a specimen is to be collected through intact skin, cleanse the skin first. For example, use 70% alcohol followed by iodine solution (1 to 2% tincture of iodine or 10% solution of povidone-iodine). Re-cleaning of area with 70% alcohol should be done before specimen collection.
- Before collection of the specimen, consider the risk/benefit ratio of the collection procedure and give appropriate instructions to the patient.
- Collect specimens in a sterile, screw-cap, leak proof containers with lids that do not create an aerosol when opened.
- Transport all specimens to the laboratory immediately after collection.

5.3.3 Equipment Requirement

Sterile containers /swabs/ transport medium/ 70% alcohol/ povidone-iodine solution/ Zip lock bags/ test request forms/ sample rejection criteria charts/documentation facilities and guidelines as per the requirement.

5.3.4 Specimen Collection Procedure

- All clinical specimens must be collected in clean sterile containers, which must be properly sealed. The outer portion of the container must not be soiled.
- Ensure no leakage/breakage of containers.

- Optimal specimens are aseptically obtained fresh pus, fluid, or tissue that is rapidly and safely transported to the laboratory. Direct aspiration into a syringe is recommended. Swabs should not be used in cases where fluid/tissue can be obtained.
- Swabs without transport medium are not satisfactory since they allow drying of the specimen and loss of viability of the microorganism. All specimens should be transported to the laboratory in a sealed zip lock bag.
- The following are **reasons for rejection** of specimens:
    - Inappropriate requisition/incomplete test requisition form
    - Unlabeled/mislabeled sample
    - Incorrect container
    - Incorrect volume
    - Obvious foreign particle contamination
    - Specimen unsuitable for culture request; i.e. anaerobe request from aerobic transport
    - Only one swab for multiple test requests
    - Leaking container
- **Anaerobic cultures** are best collected by aspirating abscess fluid with a sterile syringe and needle. The aspirated fluid can be injected into an evacuated tube anaerobic specimen collection container.
    - The submission of swabs for anaerobic culture is discouraged, but if swabs must be used, they should be placed immediately into anaerobic transport tubes.
    - Anaerobic bacteriology is time-consuming and expensive. Thus, it should be done only on appropriate and properly collected and transported specimens. Specimens should be delivered to the lab as soon as possible.

5.3.5 Specific Collection Guidelines

a. Collection of Wound Swabs

- Gentle cleansing of a skin wound with sterile saline prior to sample collection is recommended to reduce commensal flora contamination.
- Remove surface exudate by wiping with sterile saline.
- Tissue or aspirate is always superior to a swab specimen. If swabs must be used (aerobic culture only), collect two, one for culture & one for Gram staining.
- Aspirate if possible or pass a swab deep into the lesion to firmly. Sample the lesion's fresh border. The samples of the base of the lesion and abscess wall are most productive.
- Purulent exudates must be expressed onto swabs.
- Place the swab into a sterile container containing appropriate transport media (as per laboratory protocol).
- Label the container with patient's full name, date of birth or unique ID number, source of specimen and date and time of collection.

- Maintain swabs at room temperature or incubate wherever it is collected and submit to the laboratory immediately (within 24 hours of collection).
- Deep wound specimens (aspirates) are optimal as the predictive value of superficial swabs is low. Investigation of deep wounds for anaerobes requires a special anaerobic collection kit, available from the laboratory.

b. Collection of Eye Swabs

- Collect before topical solutions/antibiotics or anesthetics are applied.
- Swab pus or purulent discharge taken from the surface of lower conjunctival sac and inner canthus(angle) of the eye.
- Both eyes should be cultured separately.
- Place the swab into into a sterile container containing appropriate transport media (as per laboratory protocol).
- Label the container with patient's full name, date of birth or unique ID number, source of specimen and date and time of collection.
- Maintain swabs at room temperature or incubate wherever it is collected and submit to the laboratory immediately (within 24 hours of collection).

c. Collection of Ear Swabs

- Should be done by an otolaryngologist using sterile instruments.
- For diagnosis of external otitis, the external ear should be cleansed with a mild germicide e.g. 1:1000 aqueous solution of benzalkonium chloride to reduce contamination by skin flora.
- Place the collected swab into a sterile container containing appropriate transport media (as per laboratory protocol).
- Label swab with patient's full name, date of birth or unique ID number, source of specimen and date and time of collection.
- Maintain swabs at room temperature or incubate wherever it is collected and submit to the laboratory immediately (within 24 hours of collection).

d. Collection of Throat and other upper respiratory tract Swabs

- Sterile, Dacron or Rayon Swabs with Plastic Shafts are Suitable.
- Cotton swabs should never be used for culture because fibres contain fatty acids on their surface which inhibit growth of *Bordetella spp*. Cotton swabs are unacceptable as it may interfere with staining of AFB.
- Throat swabs are adequate for recovery of *Streptococcus pyogenes, adenoviruses, herpes viruses, Corynebacterium diphtheriae, Mycoplasma, Chlamydia,* and *Candida spp*

- Nasopharyngeal swabs are best suited for recovery of Bordetella pertussis, Neisseria spp., respiratory syncytial virus etc.
- Moist swabs can be transported to the laboratory within 2-4 hours of collection. In case of delay, a transport medium is required to maintain viability of pathogens and prevent overgrowth of contaminants.
- Swab the tonsillar area and/or posterior pharynx by trained medical staff.
- Place the swab into a sterile container containing appropriate transport media (as per laboratory protocol).
- Label the container with patient's full name, date of birth or unique ID number, source of specimen and date and time of collection.
- Maintain swabs at room temperature and submit to the laboratory immediately.
- If there is any delay in transportation, the swab should be held at 2-8 degree Celsius
- For suspected viral pathogens, the swab should be transported in viral transport medium.

e. Collection of Rectal swab

- Carefully insert a swab approximately 1 inch into rectum through anus beyond the anal sphincter.
- Gently rotate the swab to sample the anal crypts.
- Faeces should be visible on the swab for detection of diarrheal pathogens.
- Transport should be in unpreserved and transport time should be $\leqslant$ 2 hrs, at room temperature in Holding medium: $\leqslant$24 hrs, at room temperature.
- Reserved for detecting N. gonorrhoeae, Campylobacter, Herpes Simplex Virus, and anal carriage of group B Streptococcus and other beta-hemolytic Streptococci, or for patients unable to pass a specimen.

f. Collection of Genital Swab Cultures include Organism/Syndrome Urethral specimen collection

- Urethral discharge may occur in both males and females infected with pathogens e.g. Neisseria gonorrhoeae and Trichomonas vaginalis.
- Swabs used are made of cotton or rayon treated with charcoal to adsorb material toxic to gonococci and wrapped tightly over one end of a thin wire shaft.
- Calcium alginate swabs are generally more toxic for Herpes simplex virus, gonococci, chlamydiae and mycoplasmas.
- Dacron swabs are least toxic and recommended for viral specimens.
- To obtain a urethral specimen, a swab is inserted approximately 2 cm into the urethra and rotated gently before withdrawing. Two swabs are required. One for microscopy and other for culture.
- When profuse urethral discharge is present, particularly in males, the discharge may be collected externally without inserting a sampling device into the urethra.

I Cervical/vaginal specimen collection

- Swabs are handled as previously described for urethral swabs for isolation of Trichomonas and gonococci.
- Endocervical specimens are obtained after the cervix has been exposed with a speculum, which allows visualization of vaginal and cervical architecture, after ectocervical mucus has been adequately removed.
- The speculum is moistened with warm water and a small, nylon-bristled brush or Cytobrush may be used to ensure that cellular material is collected.
- Genital tract infections caused by sexually transmitted agents in children (preadolescents) are most often the result of sexual abuse. Because of medico-legal implications, the laboratory should treat specimens from such patients with extreme care.
- Transport swabs collected for isolation of gonococci may be transported to laboratory in modified Stuart's or Amies'charcoal transport media and held at room temperature until inoculated to culture media.
- Swabs for isolation of chlamydiae and mycoplasmas are transported in specific transport media containing antibiotic. Specimens must be transported on ice and specimens transported at room temperature should be processed within 15 minutes of collection. Specimens can be stored at 4ºC for upto 24 hours.

ii. Uterine

Culdocentesis is performed after decontamination of vagina with povidone iodine or aspirate into syringe through IV type catheter passed through the cervical opening under direct visualization. Vaginal or cervical swabs are collected.

g. Abscess

Needle and syringe aspirate of closed abscess after decontamination of surface. Swab is obtained from surface of abscess or swab after incision and drainage.

h. Decubitus Ulcer

A swab is not the specimen of choice. A tissue biopsy sample or needle aspirate is the specimen of choice. The surface should be cleansed with sterile saline. If a biopsy sample is not available, aspirate inflammatory material from the base of the ulcer. Transport mode should be sterile tube/container (aerobic) or anaerobic system (for tissue). Since a swab specimen of a decubitus ulcer provides no clinical information, it should not be submitted.

I Others

Joint fluid, spinal fluid, blood and biopsy tissue collected with care by specialists as per protocol.

j. Surveillance Specimens

i. Appropriate Specimens for Screening are:

- MRSA: Nasal Swab, Rectal/ Perineal Swab, Axillary Swab, Wound Swab, Swab from sites previously positive for MRSA.
- VRE: Rectal Swab, Perianal Swab, Swab from sites previously positive for VRE, Urine, Stool. Nasal swabs are not appropriate for VRE.
- ESBL and CPE/CRE (Carbapenemase producing bacteria): Rectal Swab, Wound Swab, Swab from sites previously positive, Urine, Stool, Endotracheal secretions or samples from other exit sites
- Group A Streptococcus: Throat Swabs, Nasal Swabs, Rectal Swabs, Vaginal swab.

ii. Swabs and Skin Lesion Swabs

- Record the target organism as well as body site on the requisition. E.g. Rectal swab for VRE.
- Swabs should be collected and transported to the laboratory in appropriate transport medium provided by the laboratory .
- For MRSA, VRE and Group A Streptococcus, a separate swab should be collected for each target organism requested.
- Urines, stools, sputum, endotracheal secretions or samples from other exit sites should be collected in sterile containers. Stools in Cary Blair medium are also acceptable.
- Refrigerate urine, stool and sputum specimens at 2-8ºC and submit to the laboratory immediately after collection (within 30 minutes).
- Maintain all other specimens at room temperature and submit to laboratory WITHIN 24 hours of collection.

5.4 Physician Collected Samples (Lavages/Cavity Fluids/ Histopathology/Cytology)

Samples are collected for cell counts, cytology, biochemistry and microbiology.

Equipment: Sterile, leak proof container/collection equipment specific to each activity

5.4.1 Gastric Wash or lavage

- Collect in early morning before patients eat and while they are still in bed;
- Introduce a nasogastric tube to the stomach
- Perform lavage with 25-50 ml of chilled sterile, distilled water

- Recover sample and place in a leak proof, sterile container

5.4.2 Lower Respiratory (Broncheoalveolar Lavage, brush or wash, Endo Tracheal Aspirate etc.)

- Collect washing or aspirate in a sputum trap
- Place brush in sterile container with 1 ml saline
- A total of 40-80 ml of fluid is needed for quantitative analysis. For qualitative analysis of brushings, place brush into 1.0 ml of saline.

5.4.3 Cavity Fluids:

CSF/Pleural/Pericardial/Peritoneal/Synovial//Amniotic/Paracentesis

- These should be received in sterile containers without leakage and with appropriate sample details.
- Laboratory technician must receive information about time of collection, appropriate transportation and/or storage.

Note: Amniotic and culdocentesis fluids should be transported in an anaerobic system and need not be centrifuged prior to Gram staining. Other fluids are best examined by Gram staining of a cyto-centrifuged preparation.

5.4.4 Histopathology

A. Routine Histopathology

- All specimens for routine histological examination MUST be collected into wide necked containers with 10% neutral-buffered formalin solution.
- There MUST be sufficient formalin in the container to completely cover the specimen. (Ideally there should be at least 2-3 times the volume of fixative in ratio to the size of the specimen to ensure adequate fixation).
- Refrigeration (2-8 degree C) is not needed and should be avoided so as not to slow down the chemical reaction
- The later must be labeled with a 10% formalin hazard label.
- If the volume of fixative does not cover the sample, or the sample is squeezed into a container that does not allow enough formalin, add more formalin or transfer to a larger container
- Specimens MUST NOT be placed into any other solution or into a dry container (except frozen section requests), as irreversible deterioration of the specimen will take place, making accurate microscopic interpretation impossible.
- Large surgical resection specimens should not be sliced or opened by the surgeon, but sent directly to the laboratory without delay.
- Small fragile specimens (bone marrow, Tru-cut liver or kidney) can be wrapped in a gauze envelope so that they do not disintegrate during transport

- All samples should be placed in a well-sealed leak proof bag containing enough absorbent material for the volume of formalin.

B. Frozen section

- Specimens for frozen section MUST be placed in a suitably-sized DRY specimen container and transferred immediately to the Histology Department by hand.
- Tissue for frozen section must be handed directly to a Medical Scientist or Consultant Pathologist.

C. Muscle Biopsy

Muscle specimen should be wrapped in saline dampened gauze and placed into an empty white topped Universal container. Do not over soak the gauze as this causes problems with ice crystal artifact formation during subsequent processing. Make sure the specimen is reasonably loose within the container. Take care not to squash the specimen. Do not send the sample on ice.

D. Renal Biopsy

Two specimens should be taken: one into 10% buffered formalin and the other into Zeus fluid. Both fixatives should be available for collection, prior to taking the biopsy

Trans-bronchial specimens are collected directly into the preservative solution by the Bronchoscopy Team at the time of the procedure.

5.4.5 Cytology

A. Routine Non-Gynaecological Cytology

a) Fluids

- Pleural, Peritoneal and Cyst Fluids: Up to 25- 100 ml of fresh fluid should be sent to the laboratory in a sterile, universal container.
- Cytology samples may be stable up to 48 hrs under refrigeration. NABL recommends retention up to 24 hrs at 4 degree Celsius
- Drainage bags of fluid must not be sent to the laboratory. Transport of such bags poses a serious infection risk to staff and public within the hospital.
- Sputum: Use a sterile, universal container to collect the first deep cough specimen produced in the morning, or a sample taken after physiotherapy. The sample should be collected before breakfast or teeth cleaning. Samples comprising predominantly saliva are inadequate for cytological investigation of the lower respiratory tract. Sputum cytology should not be used as a screening investigation. It should be limited to individuals in whom a histological or cytological diagnosis is desired but in whom bronchoscopy is inappropriate or unsuccessful. Ideally, the test should be carried out on three consecutive days, with each specimen being delivered to the lab as soon as it is taken.
- Urine: 20ml of urine in a sterile, universal container will be sufficient. For best cell yields, the sample should be collected at the beginning or end of voiding urine. The first morning sample is less suitable than other times as cells in the low pH and

hypertonic environment undergo degenerative changes making cytologic assessment difficult. It is extremely important that no alcohol or any other fixative is added to the specimen.

b) Fine Needle Aspiration Cytology (FNAC)

- Physicians / Pathologists prepare FNA slides at the hospital/ lab/ radiology department by prior arrangement, and return the sample(s) to the laboratory for processing.

B. Gynecological Cytology: Pap Smears

Acceptable specimens for the Pap test include cervical, cervical and vaginal, endocervical, vaginal and/or vulvar scrapings or brushings and must be labeled accordingly.

Specimens may be collected as conventional smears or Liquid Based Cytology for cell enrichment

The quality of the test results depends upon proper collection, preparation and transport of the specimen.

Patient Clinical History should be provided to ensure thorough and complete interpretation and must include; specimen source, birthdate, date of last menstrual period (LMP), gynecological surgery and procedures, whether patient is pregnant or post-partum, current hormonal therapy, presence of an IUD, past or present neoplasms, chemotherapy, radiation therapy, abnormal bleeding, previously abnormal Pap smear(s)

Specimen Collection

- Instruct patient to avoid douching 24-48 hours before examination.
- Avoid collection of samples during a patient's menstrual period.
- If patient has had a hysterectomy, submit smear from vaginal apex.

a) **Liquid Based Cytology:** Scrape the cervix circumferentially at squamo-columnar junction with a plastic spatula. Remove and rinse the spatula as quickly as possible into the LBC vial by swirling it vigorously 10 times in the solution. Discard the spatula. Gently insert the cytobrush into the cervical os until only the bottom-most fibers are exposed. Slowly rotate ¼ or ½ turn in one direction. Do not over-rotate. Remove the brush and rinse in the LBC vial by rotating the brush in the solution 10 times while also pushing against the vial wall. Swirl the brush vigorously to further release the material. Discard the brush. Store the sample at room temperature.

b) **Conventional Pap smear:** Cervical scrape and brushing is recommended. Except for evaluation of maturation index and detection of vaginal adenosis, one slide will be adequate. For routine Pap tests, both the scraping of the squamo-columnar junction and endocervical brushing can be spread onto the same slide.

Label the glass slide with the patient's last name, first name and medical record number (or date of birth) using a graphite pencil (do not use ink, wax or crayon). Gently remove excessive secretion or blood at the cervical Os with cotton swab and discard. Scrape the ectocervix circumferentially with a cervical spatula at the squamo-

columnar junction and spread the material evenly onto one half of the labeled slide. Gently insert an endocervical brush into the cervical Os. Rotate, then remove the brush and spread material evenly on the other half of the slide. It does not matter if the two samples mix together. Fix the slide immediately with spray fixative. Spray at an angle of 45 degrees to avoid cellular artifacts. Allow spray-fixed slide to dry completely and place into a slide holder.

c) **Vaginal Sample:** Obtain the cervical sample as described above and spread it on the slides. Before fixing, obtain a vaginal scraping from the posterior fornix with a cervical spatula. For patients that have had a hysterectomy, collect a sample from the vaginal apex; spread the material on a labeled slide and spray fix immediately.

d) **Vulvar or Vaginal Lesion Sample:** Collect sample directly from the lesion and submit as indicated above.

e) **Vaginal Adenosis:** Collect a scraping from each quadrant of the upper vagina and submit separately from cervical samples. Vaginal specimens should be collected before cervical samples are obtained and the area to be sampled is first swabbed to remove any contaminating secretion from the cervix.

f) **Maturation Index for Hormonal Evaluation:** Microscopic evaluation of vaginal scrapings is no longer recommended for evaluation of hormonal status. In the event it must be performed, collect a sample from the upper ⅓ of the lateral vaginal wall and submit as indicated above.

Note: HPV testing has not been approved for vaginal samples.

Unsatisfactory Results: The following conditions may render a Pap test unsatisfactory or interfere with cytologic examination: improper fixation or drying of a smear before fixation, failure to obtain adequate cellular sample, excessive use of lubricating jelly on the vaginal speculum, excessive mucus, blood, or purulent exudate, an inflammatory reaction may invalidate hormonal evaluation.

Reasons for rejection of specimens include: Improper labeling of specimen container or requisition, incomplete or missing requisition and prepared slides received broken beyond repair (for conventional Pap smears).

Labeling: Specimen containers MUST be labeled in such a way so as to provide an unequivocal link to the patient. Best practice dictates that labeling should take place in the presence of the patient, immediately after sample collection. The essential information MUST be documented, in a legible manner, on the specimen container(s). Large sample containers MUST be labeled on the pot AND on the lid.

Chapter 6

Field Sampling in Outbreaks/Epidemics

6.1 Learning Objectives

At the end of this chapter, the learners should be able to define :

a) Investigation and steps of approach in case of epidemics
b) Role of laboratory in field investigations
c) What samples to collect
d) Process of Sample collection, transportation and storage
e) Safety measures
f) High risk activities

Note: Guidelines for Specimen Collection and Transport for Laboratory Testing during Outbreak situations: IDSP Guidelines is given as Annexture K

6.2 Investigation of an Epidemic:

It calls for a prompt and thorough investigation of the cases to uncover the factors responsible and to guide in management of cases an advocating control measures to prevent further spread.

- The **objectives** of an epidemic investigation are:
  1. To define the magnitude of the epidemic in terms of time, place and person.
  2. To determine conditions and factors responsible for its occurrence.
  3. To identify the cause, source of infection, and modes of transmission to determine measures necessary to control the epidemic.
  4. To make recommendations to prevent recurrence.

- **Steps**
  1. **Verification of Diagnosis:** By clinical examination of a sample of cases. Laboratory investigations are most useful for confirmation of diagnosis, wherever applicable.
  2. **Confirmation of Existence of an Epidemic:** By comparison of disease frequencies during the same period of previous years. Often, no such comparison is required e.g. in common-source epidemics of cholera, food-poisoning and hepatitis A.
  3. **Defining the Population at Risk:** by obtaining map of area and counting the population.
  4. **Rapid Search for all Cases and Their Characteristics:** By medical survey, case sheets.
  5. **Data analysis:** Using classical epidemiological parameters – time, place and person.
  6. **Formulation, testing and evaluation of hypothesis:** To prevent further transmission.
  7. **Report preparation:** Should be complete and convincing.

6.3 Role of Microbiology Laboratory:

Laboratory works in tandem with the infection control committee by (Provision of sample collection equipment and culture/transport media in labs of affected areas)

a) Establishing the identity of pathogen causing epidemic by isolation in culture for other molecular/rapid tests

b) Preservation of isolates

c) Culture of possible reservoirs (patients, personnel or the environment)

d) Performing typing of strains to establish relatedness between isolates of the same species: The ideal system for typing microbial strains (phenotypic or genotypic) should be standardized, sensitive, stable, readily available, inexpensive, applicable to a wide range of microorganisms, and field tested in other epidemiological investigations.

6.4 Path of workflow is maintained as follows:

6.4.1 Correct Identification of Cases/Carriers: It is important to have case definitions.

- Each patient sample or each environmental sample taken should be given a unique identifier number and accompanied by a field data sheet.
- All specimens taken from that source should be marked with that unique identifier as well as any other numbers needed to identify the particular specimen.
- This identifier should be used on all documentation concerning the specimen from that source.
- Specimen tubes should also be marked with information about the type of specimen in the tube and the date when the specimen was taken.
- All specimens must be accompanied by a duly filled case referral form.

6.4.2 Correct Sample Collection:

- All steps of sample collection are followed to avoid inappropriate or inadequate sample. Sample rejection criteria must be applied unless precious sample (e.g. cadavers).
- A checklist must be used for the various stages of taking, handling and shipping specimens. This should be filled in for each batch of specimens and kept for reference. Note that the checklist covers a wide range of activities from taking the specimen to activities such as taking aliquots from specimens (sub-sampling) which may occur before the specimen is sent to the reference laboratory.
- For each type of specimen two specimens should be taken in separate specimen tubes on each occasion that sampling is undertaken. One can be used for immediate analysis and the other retained for reference purposes, retesting, etc.

6.4.3 What Specimens to Collect from Suspect Cases: Varies from case to case as per the guidelines.

- **Upper Respiratory Tract (e.g. detection of influenza viruses):** Posterior-pharyngeal (throat) swabs are currently the highest yield upper respiratory tract specimen for detecting e.g. H5N1. Nasopharyngeal swabs, nasal swabs with nasal secretions (from the anterior turbinate area) or nasopharyngeal aspirates or swabs are also appropriate specimens.
- **Lower Respiratory Tract:** Sputum is preferred e.g. pneumonia. If the patient is intubated, take a tracheal aspirate or collect a sample during bronchoalveolar lavage.
- **Blood for Smear:** e.g. Malaria, Filaria
- **Blood:** Serum (acute and convalescent if possible)e.g. serological tests for dengue, chickungunya.
- **Blood for culture:** e.g. in brucellosis
- **Stool/Rectal Swab:** especially if the patient has diarrhea/food-poisoning. E.g. cholera, toxin detection in Staphylococcal food poisoning

- **CSF** if meningitis is suspected and a spinal tap is to be performed for diagnostic / therapeutic purposes.
- **Skin Scrapings:** e.g. Meningococcemia
- **Body Fluids:** e.g. peritoneal, pleural fluid
- **Urine:** e.g in Leptospirosis (dark-field microscopy)
- **Genital discharge:** e.g. Syphilis, Gonorrhea

6.4.4 Correct Sample Transportation and Storage

- Sample should be transported at the earliest. Triple layer packaging must be done.
- Appropriate steps to be followed to avoid any deterioration or contamination of samples e.g. refrigeration of urine sample in case of more than 4 hours of delay or using preservative (boric acid).
- Storage of specimens is followed as per the specimen and agent specific guidelines.
- The packing of specimens and their shipment to external laboratories by air is complex and is governed by international and national regulations and operator variations. Readers are also referred to the WHO document Guidance on regulations for the Transport of Infectious Substances.
- International air transport of human specimens known or suspected to contain the agent or of specimens from infected animals must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations (Infectious Substances Shipping Guidelines). However be aware that specimens shipped by air need to be transported to and from the airport(s) (e.g. by road or by rail) and that different regulations may govern transport by such means — information should be obtained from the ministry of health or ministry of agriculture/chief veterinary office (for animals specimens)
- There are two categories covering shipment of specimens by air:
  i. Category A covers infectious substances that are capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals e.g. avian influenza.
  ii. Category B covers all other infectious substances that are not included in Category A.

6.4.5 Shipment of Frozen Specimens

i. Specimens that are to be shipped by air can be preserved either in dry ice or in liquid nitrogen.

ii. IATA regulations require that both types of shipments must be consigned by trained shippers.

iii. Dry ice and liquid nitrogen both give off gases that can cause asphyxiation and should only be handled in well ventilated areas. In addition any containers in which they are shipped must be able to vent evaporated gases to the air to avoid the risk of explosions.

6.4.6 Correct Sample Processing for microscopy, culture, serology, molecular or other rapid tests as approved standard guidelines for identification and typing of pathogens.

i. Classic phenotypic techniques are: dependent on phenotypic characters of pathogens e.g. biotyping, use of antibiograms, serotyping, bacteriocin, bacteriophage typing.

ii. Genotypic typing: includes analysis of genetic material as in molecular methods e.g. PCR, Plasmid analysis and restriction endonuclease analysis of chromosomal DNA, Pulsed Field Gel Electrophoresis (PFGE) etc

6.5 Appropriate Safety Measures

Appropriate safety measures must be undertaken. In general, PPE should include:

a) Latex gloves (or equivalent if allergic)

b) Mask

c) Goggles or a face shield

d) Gown

e) Head covering

Ensure appropriate safety measures including hand hygiene, general safety measures, appropriate biomedical waste management and infection control practices.

6.6 High Risk Activities

It includes such as post mortem examination of a confirmed or strongly suspected human case, capture of birds in poultry sheds or on farms, euthanasia of infected or potentially infected birds or other animals, and procedures such as decontamination in an intensive agriculture system should only be conducted in a full body cover-all, with easily cleaned waterproof boots, heavy duty rubber gloves and eye protection.

1. Microbiology laboratories as per government rules are also obliged to report certain notifiable diseases to public health authorities e.g. Cholera, Malaria, Chickungunya, Dengue etc

Chapter 7

Sample Receipt (Accessioning), Acceptance/ Rejection Criteria, Processing

7.1 Learning Objectives

At the end of this chapter, the learners should be able to describe:

a) The process for receiving the samples from OPD, wards & referral
b) How to act on missing or compromised samples
c) The acceptance and rejection criteria of the samples
d) Specimen preparation before testing

7.2 Sample Receipt (Accessioning)

Equipment

- Laboratory Register Book
- Pens; black and red
- Fine tip Markers
- Gloves
- Laboratory Coat
- Sample Racks
- Accessioning Register/ Computer for Accessioning
- Disinfectantant
- Rejection Forms
- Documentation procedures (e.g. reconciliation forms/register) for outside samples

Purpose: To receive, check, verify the integrity of sample, prepare and deliver samples to the respective work area

Procedure

- **In-House Samples**

  The laboratory procedure for sample reception should follow standard essential steps:

  - All samples should be accompanied with test request forms;
  - Samples should be unequivocally traceable, by request and labeling, to an identified client by unique identification number and laboratory number;
  - All samples received should be recorded in relevant registers. The date and time of receipt and/or registration of samples should be recorded in the respective register;
  - Authorized laboratory technicians/counselors evaluate the received samples to ensure that they meet the sample acceptance criteria relevant for the requested examination(s). Sample rejection criteria should be followed by laboratory staff;
  - All the samples should be treated equally except for the physician requested urgent specimen or in case of needle stick injury.
  - Department-wise check lists may be generated for Biochemistry, Haematology, Clinical Pathology, Serology, Microbiology, Histopathology, and Cytology and specimen referrals. The samples are to be separated department –wise, centrifuged or processed as per protocol (see below) and delivered to the respective departments.

- **Samples Received from Outside:**

  - All samples should be accompanied with test request forms;
  - The samples must be shipped to the laboratory under conditions that are appropriate for subsequent processing, culture and testing;

- Unpack the patient samples and the requisition forms. Match each sample with the corresponding request form and make sure that the identifiers on the sample containers match those on the request form;
- If there is an inconsistency between the sample label and request form, a procedure should be available to deal with these discrepancies. A reconciliation form is suggested. Copies of the request and reconciliation forms should be archived;
- The date and time that the samples were received at laboratory and the initials of the personnel handling the samples, sample appearance and volume are noted on the lab test requisition form;
- Record the sample laboratory number and the patient's information in the laboratory register book in which subsequent monitoring will be done;

7.3 Guidelines for Specimen Rejection:

Sample rejection criteria are designed to prevent inaccurate data and ensure the quality of testing.

- **Unlabelled Samples**
  Specimen types (blood, urine, swabs, sputum, stool, etc.) which can be easily re-collected and cannot be identified with certainty, should be rejected and fresh sample recollected.
- **Incompletely Labelled (Mislabelled) Samples**
  Specimens which are labelled incompletely need to be verified satisfactorily by the technician with the help of the test request form. If that is not possible, the collection is to be repeated;
- **Incorrect Container or Preservative:**
  Specimens received in an incorrect container, or with /without appropriate preservative/ anticoagulant, which would invalidate results, will require recollection;
- **Insufficient Samples**
  All insufficient specimens will need to be re-collected;
- **Excessive Delay** in specimen transport or improperly transported (ie, not on ice when required)
- **Specimen Contaminated** with biological hazardous material
- **Sub Optimal Samples :** All haemolysed blood samples should be recollected. If the EDTA blood shows clots, recollection is to be done. If cold chain is not maintained during transport the sample should be recollected.
- **Leaking Containers.**
  Duplicate samples received on the same day for advised consecutive day examination i.e. multiple stool, sputum specimen (if not indicated) should not be accepted.
- **Compromised Samples in Critical Cases**
  In case of compromised samples in critical cases where replacement of sample is not possible, the lab shall perform the test and release the reports with suitable remarks to the patient/requesting physician. This applies particularly to histopathology samples with inadequate formalin and insufficiently sized containers

7.4 Sample Preparation

- **Purpose:** To enable optimum reporting and to avoid pre-analytical errors

- **Procedure**
  - Never centrifuge glass tubes above 2200g in a horizontal head (swinging bucket) centrifuge or above 1300g in fixed angle centrifuge heads, as breakage may occur. The clotting of a sample must be fully completed before centrifugation of serum tubes.
  - All samples collected in serum tubes (Yellow with gel separator/ Red without gel separator) should be allowed to clot for a minimum of 30 minutes to prevent latent fibrin formation and are centrifuged at 3500 rpm for 10 minutes or as recommended by the manufactures for biochemistry analysis.
  - The centrifuging should be done within 2 hours of sample collection.
  - In case separator/gel tubes are not used serum/plasma should be alliquoted to a secondary collection within 2 hrs of blood collection. Secondary tube should be proper labelled to reflect all patient information present on the primary tube.
  - All samples in fluoride (Grey) tubes are to be centrifuged at 3000 for 5 minutes or as recommended by the manufactures.
  - All EDTA samples for CBC and ESR are processed according to their respective SOPS. No centrifugation should be done
  - For ESR, 4:1 dilution of well mixed EDTA blood and 3.8% sodium citrate is required. Should not be centrifuged. Alternatively, citrate tubes (Black) may be used. This may be used in the evacuated method also.

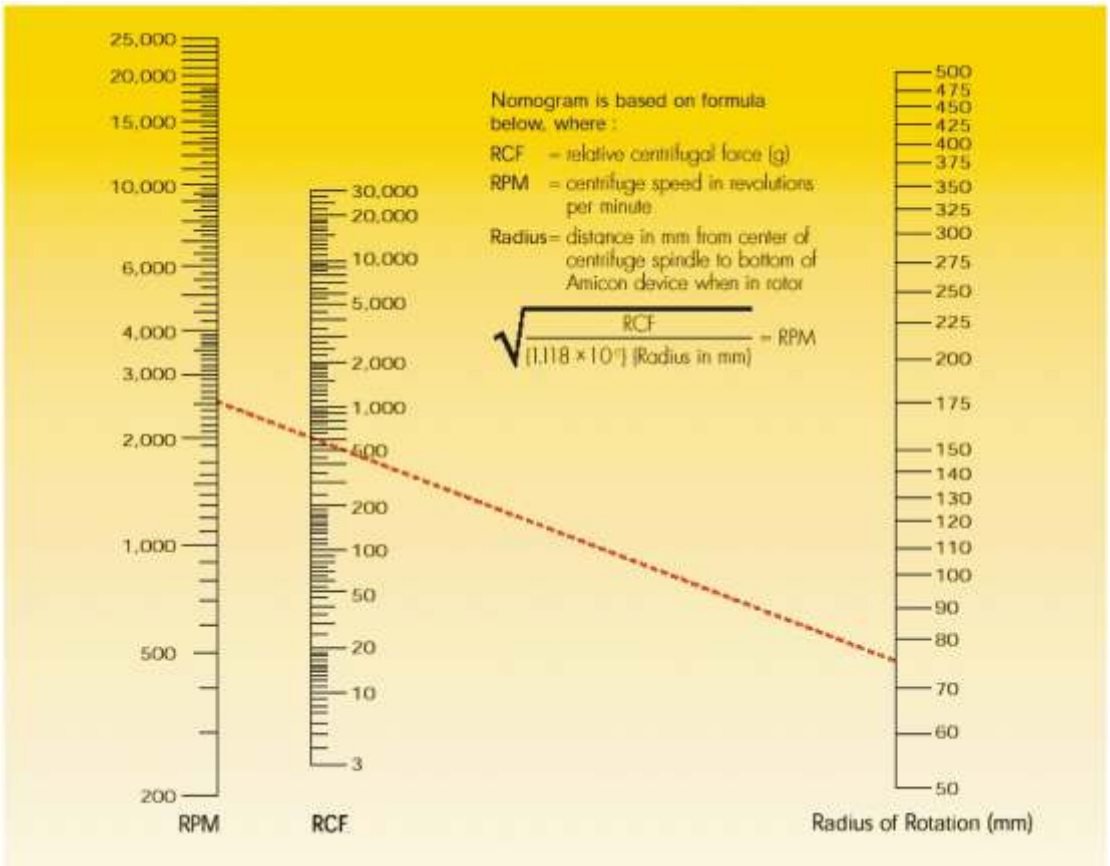


Figure 30: Nomogram

Urine samples are centrifuged at 1500 for 10 minutes for microscopic examination, after the physical and chemical examinations are done.

- For therapeutic drugs monitoring tests or toxicological analysis collection is made in plain tubes or gel tubes where manufactures has data to prove compatibility for the drug being analyzed. Do not invert the tube. Let the tube stand in the rack for 30 minutes till blood clots. Centrifuge at 3500 rpm for 10 minutes or as per manufacturer instructions.
- For coagulation studies the samples to be collected in 3.2%sodium citrate tube. All other anticoagulants are unacceptable. The sample should be centrifuged at 2000 g or 4000 rpm for 15 minutes to obtain platelet poor plasma. Each lab has to standardize the centrifuge speed so as to get the plasma platelet count of $<10,000/\mu L$.

Converting RPM to RCF

Conversion of Revolutions per Minute (RPM) to Relative Centrifugal Force (RCF) expressed in 'g' depends on the radius of the centrifuge. It can be calculated by reference to the normogram, (see below)

$$RCF = 118 * 10^{-7} * r * N^{-2}$$

Where r is the radius (cm) and N is the speed of rotation (RPM).

Low spun, platelet rich plasma 150 to 200 g for 10-15 minutes

High spun plasma for 1200 -1500 g for 15 minutes

Packing of cells: 2000-2300 for 30 minutes

Measure the radius (cm) from the center of the centrifuge rotor to the end of test tube carrier. Obtain the relative centrifugal force necessary for the application. A straight line connecting the value of the radius with the relative centrifugal force (g) value will enable the speed of the rotor (rpm) to be read off of the right column.

After the samples that are segregated and prepared are tested according to their respective SOPs

Chapter 8

Sample Referral and Transportation

8.1 Learning Objectives

At the end of this chapter, the learners should be able to understand:

a) To understand the need for specimen referral
b) To ensure appropriate selection of referral laboratory
c) To understand specimen preparation before transportation
d) To understand chemical safety requirements
e) To follow safe transportation and safety regulations
f) To ensure adequate procedures for receipt of report from referral laboratory

8.2 Need for Specimen Referral

1. When tests are not available at the laboratory (e.g. in peripheral facilities/in the setting of epidemics)
2. To allow performing specialized tests at central facility (without unnecessary patient referrals, ensure good quality of results and better patient continuity)
3. As a backup measure e.g. equipment breakdown, non-availability of laboratory personnel at facility, stock outs
4. As a part of national EQAS programme/other inter laboratory comparisons
5. For confirmation of test results

8.3 Procedure for Selecting a Referral Lab

Prerequisites of the referral lab should be ascertained by studying the detailed procedure of tests, performance specifications and adequacy to meet the requirements of the referring lab. This should be ascertained by qualified personnel. Periodic monitoring and evaluation of these labs should be done and records maintained.

Institution/ Medical centers should establish and implement a documented procedure for monitoring the transportations of samples to ensure they are transported:

- **The right timeframe:** Within a time frame appropriate to the nature of the requested examinations and the laboratory discipline concerned (the serum samples are transported to the laboratory at the earliest in triple layer packaging);
- **The right temperature:** Within a temperature interval specified for sample collection and handling and preservatives should be used (cold chain is maintained usually with the help of ice packs/ice box);
- **The right packaging:** In a manner that ensures the integrity of the sample and the safety for the carrier, the general public and the receiving laboratory, in compliance with established requirements (triple layer packaging with biohazard symbol on the outside of the packaging with maintenance of cold chain). Regardless of the sophistication of the packaging materials, it is possible to properly package and ship specimens for referral testing.

8.4 Specimen Preparation before Transportation

1. Blood specimens collected for potassium and/or glucose testing must be separated from the red cells within 4 hours of collection or inaccurate test results may occur (ref:CLSI H18-A2). Falsely elevated potassium levels or falsely decreased glucose levels may result when the specimen remains uncentrifuged for more than 4 hours after collection
2. Contact time of less than two hours is recommended for samples for ACTH, cortisol, Catecholamines, lactic acid, and homocysteine.
3. Specimen collected for some tests need to be kept in crushed ice immediately after collection, these are: Catecholamines, ammonia, lactic acid, pyruvate, gastrin, and parathyroid hormone (PTH)

4. Coagulation guidelines Samples should be transported as per current guidelines, non-refrigerated at ambient temperature (15–22°C) in as short a time as possible. Ideally, testing for routine coagulation tests like the PT and the APTT should be accomplished within 4 hours of collection, although allowable tolerances may be greater than this. Prothrombin time (only in case of ambient temperature – however if the transport temperatures are 2-80C then even PT needs to be tested within 4 hrs). However, APTT testing for unfractionated heparin monitoring should preferably be processed within 1 hour due to the potential for heparin neutralization by release of platelet Factor IV.. Extremes of temperature (ie, both refrigerated and high) should be avoided. Delays in transport may affect in particular the labile factors (FV, FVIII), leading to prolonged clotting times and in vitro loss of factor activity. In such cases, local centrifugation and separation of plasma followed by freezing and frozen transport of the plasma should be considered.
5. Analytes like bilirubin, porphyrins are photo-labile and needs protection from direct light. They must be transported in appropriate materials, such as foil, or should be collected in a light sensitive tube otherwise results may be affected.
6. These transport conditions can be optimally monitored with use of appropriate devices which shall include a data logger.

8.5 Safety Requirements

Laboratories that mail or transport samples by air, sea, rail, and road between local, regional, and reference laboratories or between laboratories in other countries must adhere to a number of regulations. These regulations are designed to deal with transportation accidents and spills, reduce biohazards, and keep samples intact for testing.

- Regulations: for transporting samples come from several sources, including:
  - National Transport Regulations;
  - International Civil Aviation Organization (ICAO), as conveyed by the International Air
  - Transport Association (IATA);
  - Rail and Road Traffic Agencies;
  - Postal services.
  - Private courier companies may have their own requirements.
  - Compliance with industry standards and regulations is mandatory. Heavy fines maybe imposed on personnel who violate these regulations. At risk are: the safety of courier, carrier, and laboratory personnel, as well as passengers.

    The United Nations committee of experts, consisting of voting representatives from over 30 countries and non-voting advisors from various organizations, makes recommendations for the transport of dangerous goods. Many countries adopt the United Nations regulations in their entirety to stand as their national dangerous goods regulations. Some countries apply variations.
- Classification Sample transport requirements are based on the category of samples being transported.

Infectious substances are classified as Category A or Category B. There is no direct relationship between Risk Groups and Category A and B.

- **Category A:** Infectious substances capable of causing permanent disability or life threatening or fatal disease to humans or to humans and animals. These are assigned the following proper shipping name and UN number:
  Infectious substance, affecting humans, UN 2814
  Infectious substance affecting animals only, UN 2900
- **Category B:** Infectious substances that do not meet the criteria for inclusion in Category A. They are assigned the following proper shipping name Biological substance, Category B, and UN number UN 3373

  Medical or clinical wastes that contain infectious substances also need to be classified as Category A or B depending on the infectious material and whether it is present in the culture.

  Exemptions: The UN Model Regulations for the Transport of Infectious Substances includes a list of exemptions, which are samples that have a minimal likelihood that pathogens are present. They do not have the same requirements for packaging and shipping as Categories A and B

8.6 Safe Transport of Specimens (See Annexure C)

Safe transport of specimen is essential for maintaining specimen integrity, safety of personnel and environment and accurate test results.

WHO recommends triple layer packaging system for transportation of infectious substances which is as follows:

- "Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging.
- Secondary packaging shall be secured in outer packaging with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging"
- The transport box or outer packaging should be handled with care. Keep package upright in a secure in position;
- Keep and transport at compatible condition and in specified time.

8.6.1 Follow Standard Precautions during transport. These include;

a) **Hand washing** (or using an antiseptic hand-rub) after touching blood, body fluids, secretions, excretions and contaminated items;

b) **Gloves** should be used for contact with blood, body fluids, secretions and contaminated items. *Note:* Exterior of triple-packaged specimen transport containers are not considered contaminated and do not require gloves for routine handling;

c) Assure that all regulations and requirements are met when transporting samples;

d) All personnel who package or who drive transport vehicles should be trained in the proper procedures, both for safety and for good maintenance of samples;

e) When transporting locally, whether by ambulance, or by clinic or laboratory staff, it is important to maintain sample integrity. Assure that temperatures are controlled, using ice boxes or air conditioning, set an acceptable transport time, and monitor compliance.

8.6.2 Spill Cleanup Emergency Spill Kit

Routine handling is generally considered safe, but readiness for spills should be available

Disposable gown, gloves, face / eye protection, biohazard bags, disposable absorbent material, disposable cloths / paper towels, disinfectant, forceps are recommended

Personal exposure takes priority over clean up.

- Protect Yourself
- Contain the Spill and Secure the Area
- Disinfect the Spilled Material
- Clean Up the Spill Debris
- Dispose off the Material
- Clean Yourself Up

8.7 Ensuring Adequate Procedures for Receipt of Report from Referral Laboratory

- There should be a procedure for verify the reports received from referral laboratory.
- The referring laboratory must enter into a written agreement with the referral laboratory for specimen referral, transportation and receipt of results.
- The laboratory is responsible for ensuring test results being provided to the patient.
- These reports should be handed over to the patients in the original form (without alterations).

Chapter 9

Sample Storage

9.1 Learning Objectives

At the end of this chapter, learners should be able to define:

a) How specimen are to be stored

b) Appropriate storage conditions and time of storage for each specimen

9.2 Safe Storage of Specimen

- Cabinets or storage space should be available for securing patient samples and avoiding deterioration, loss or damage after examination activities.
- Appropriate facilities such as ''samples only'' or "kits only" refrigerators should be available.
- These refrigerators should be monitored using calibrated thermometers.
- There should be an indexing system for retained samples
- Specified length of time of retention of specimen as decided by the laboratory.
- Monitor the stored samples and do not keep for longer than necessary.
- Storage verification should be done with retained sample verification and if possible statistical analysis
- Sample freeze thaw cycles should be monitored.
- Access restriction to the samples is mandatory.
- Legal concerns regarding certain types of procedures, e.g. histopathology examinations may warrant longer periods of sample retention.

9.3 Temperature and Retention Time Requirements for Sample Retention

The suggested sample retention period as defined in NABL 112 is as follows.

Table 9.1 Storage conditions for the samples

Clinical Biochemistry:
1 day at 2-8˚C
Hematology:
Complete Blood Counts: 24 hours at 2-8 ˚C Coagulation screening test: <4 hrs at room temperature Hemoglobin electrophoresis and HPLC – 1 week at 2-8 ˚C or longer below -20 ˚C Bone Marrow slides – 5 years * HLA typing cell preparation – 3 days
Clinical Pathology:
Semen morphology slides – 1 week
Serology / Microbiology:
Three days at 2-8 ˚C Sample for culture: Until the final identification and antibiotic susceptibility report is issued
Histopathology:
Specimens – 30 days Slides/ Blocks – 10 years* Bone marrow aspirate and corresponding blood film and biopsy – 5 years

Cytopathology:
Fluids – 24 hours at 2-8 ˚C
Slides – 5 years
Genetics:
Blood samples for karyotyping – 6 days at 2-8 ˚C
Extracted DNA – 5 years at -20 ˚C
Extracted RNA – 5 years at -70 ˚C
Molecular diagnostic gel pictures – 5 years
Flow Cytometry:
Lysed stained samples can be re-suspended in buffered-formaldehyde solution (fixative) and stored at 2-8 ˚C until analysis.
PCR:
Blood with EDTA – up to 7 days at -20 ˚C or indefinitely at -70 ˚C RT PCR: Extracted RNA - indefinitely at -70 ˚C

* The laboratory may consider giving the original slides to its patients on specific request for obtaining second opinion or for treatment elsewhere. The laboratory shall have a documented procedure and maintain records of the same. However, attempt should be made to retain at least one representative primary slide on which the diagnosis was based for review during the follow up.

Histopathology Gross Samples:

Empty specimen containers are held in the event that the demographics need to be checked. These contain no remnant tissue or formalin. Specimen containers with remnant tissue in fixative are stored for 30 days or longer as per Lab policy. Paraffin blocks and glass slides are not discarded. These are to be retained for 10 years as per NABL 112

Chapter 10

Infection Control

10.1 Learning Objectives

At the end of this chapter, the learners should be able to understand.

a) The practices that could cause risk to healthcare workers / clients / patients / community

b) The list of infections transmitted through occupational exposure e.g. needle stick injury

c) The importance of standard precautions

10.2 Importance of Infection Control Processes

Healthcare professionals (HCP) are potentially exposed to infectious materials such as blood, tissue, specific body fluids, medical supplies/ equipment or environmental surfaces contaminated with these substances are constantly exposed to occupational hazards through exposure of per-cutaneous injury. Healthcare workers are thus at high-risk due to occupational exposure to blood-borne pathogens. Exposure can occur in a variety of ways such as needle stick or cut with sharps, contact with the mucus membrane of eyes or mouth of an infected person, contact with non-intact skin particularly when exposed skin is chapped, abraded, or afflicted with dermatitis or contact with blood or other potentially infectious body fluids.

10.2.1 What can you contract from a Needle stick?

Table 10.1 Diseases transmitted by NSI

Viral Infections	Bacterial Infections	Fungal Infections
Hepatitis B	*Brucella abortus*	*Blastomyces dermatitidis*
Hepatitis C	*Corynebacterium diphtheriae*	*Cryptococcus neoformans*
Hepatitis G	*Neisseria gonhorreae*	*Sporotrichum schenkii*
Human Immunodeficiency Virus	*Leptospira icterohaemorrhagiae*	
Simian Immunodeficiency Virus	*Mycobacterium marinum*	Protozoal Infections
Herpes simiae	*Mycoplasma caviae*	*Plasmodium falciparum*
Herpes simplex	*Orientia tsutsugamushi*	*Toxoplasma gondii*
Herpes zoster	*Rickettsia rickettsii*	
Ebola/Marburg	*Staphylococcus aureus*	Tumors
Dengue	*Streptococcus pyogenes*	Human Colonic Adenocarcinoma
Creutzfeldt-Jakob Disease	*Treponnema Pallidum Pallidum*	Sarcoma
	Mycobacterium Tuberculosis	

10.2.2 Risk of Infection Due to Sharps Injury (Percutaneous exposure) (Based on CDC statistics)

Virus	Risk
HBV	9-30%
HCV	3-10%
HIV	0.25-0.3%

10.2.3 Risk Factors for HIV/HCV/HBV Transmission after Percutaneous Exposure

Deep injury, injury with device visibly contaminated with blood, procedure involving placement of device in patient's artery or vein, hollow bore needles, high viral load in source patient, failure to take/complete post exposure prophylaxis for HIV, failure to have protective titer of anti HBS Ag antibody are the risk factors for transmission of blood borne pathogens.

10.3 Standard Precautions

Note: Consider that all patients and all samples are potentially infectious:

Table 10.2 Standard Precautions

1.	Practice effective hand hygiene procedures. Follow WHO recommended six steps of hand washing
2.	PPE: Wear gloves during blood drawing and use of gowns, masks, respirators, other personal protective equipment (PPE), wherever indicated. Wear closed-toed shoes or boots (no open-toed. shoes, sandals)
3.	Gain knowledge of spill management
4.	Avoid touching contaminated equipment. Carry out routine and proper decontamination of surfaces and instruments
5.	Practice safe disposal of sharps and infectious waste
6.	Get immunization against preventable infections including HBV
7.	Manage the needle stick injury and other occupational exposure as per procedure and take Post Exposure Prophylaxis.
8.	Manage Bio Medical Waste Management as per BMW 2016 guidelines.

10.3.1 Hand Hygiene (See Annexure D)

Most important means of preventing and controlling spread of infection is hand hygiene.

Situations that require hand hygiene (Hand washing)

- Before and after each patient contact;
- Between unrelated procedures (e.g. wound care, blood draw);
- Before putting on gloves and after taking them off;
- Before eating, going to lunch or on break;
- Before and after going to the washroom;
- Whenever hands become visibly or knowingly contaminated;
- Hand hygiene can be performed by;
- Hand washing – soap and water;
- Hand rub using gel/solution containing alcohol and/or chlorhexidine (hand washing always preferred);

10.3.2 Personal protective equipment (PPE)

All specimens should be collected using standard precautions (i.e. wearing of appropriate gloves and aprons and washing and drying of hands before and after the procedure).All blood and body fluids are potentially infectious and precautions are necessary to prevent exposure to them. All skin lesions e.g. cuts, scratches, dermatitis or other breaks are always covered with water-proof dressing before working in the laboratory. Everyone involved in providing care in the community should know and apply the universal precautions of hand decontamination, the use of protective clothing, the safe disposal of sharps and body fluid spillages. Each member of staff is accountable for his/her actions and must follow safe practices.

- Wear laboratory gown/coat.
- Inspect hands for cuts, scratches or other breaks in the skin.
- If the skin is broken, cover with occlusive bandage and wear gloves.
- Take care to avoid contaminating hands during collection of blood.
- If blood gets on gloves, these should be discarded.
- Destroy needles in a needle-destroyer and place used syringes in a puncture-resistant container. Do not recap needles. Do not remove needles from syringes.
- Seal specimen containers securely.
- Wash hands with soap and water immediately after any blood contamination and after work is completed.
- In the event of needle stick injury or other skin puncture/wound, wash the wound thoroughly with soap and running water. Encourage bleeding. Do not press/squeeze the wound.
- Post Exposure Prophylaxis guidelines should be followed.

Gloves and Aprons:

- A disposable apron and latex or nitrile gloves should always be worn when dealing with excreta, blood, body fluids, blood soiled items or whenever there is a possibility of exposure to blood.
- To be Worn while collecting or handling blood specimens, and worn while disposing laboratory waste.
- Well fitting, disposable gloves to be used and should be changed if visibly contaminated with blood.
- Removed gloves before handling door knobs, telephones, pens, performing office work and leaving the laboratory.
- Gloves should be torn before discarding avoid reuse

Masks:

- Face masks and eye protection: Must be worn as there is a risk of blood, body fluids, secretions or excretions splashing into the face and eyes. A good quality filter type should be used. It must fit the face closely and be changed if it becomes wet.

10.3.3 Spill Management

If blood/body fluid spillage has occurred (e.g. because of a laboratory sample breaking in the phlebotomy area or during transportation, or excessive bleeding during the procedure), clean it up as given below

Prepare bleach solution fresh daily and keep it in a closed container because it degrades over time and in contact with the sun.

Wear gloves

↓

Cover with absorbent material e.g. gauze/cotton

↓

Pour 1% sodium hypochlorite or equivalent disinfectant

↓

Leave for 30 minutes

↓

Wipe off the soaked absorbent material from the area

↓

Throw the wiped material into black bag*

↓

Finally, wipe the surface with 1% Sodium hypochlorite again

↓

Mop up to leave a clean area

*** Note:**

1- Black bag is mentioned here as the disinfection process renders the material harmless.

2-Putting the disinfected material in to the yellow bag can be more harmful. The yellow bags are incinerated by the operator. Hypochlorite will release chlorine on incineration.

10.3.3.1 Essential Requirements In Case Of Spill:

1. Gloves should be worn, avoid direct contact of gloved hand with spill.
2. Sweep broken glass/fractured plastic with dustpan and brush/broom.
3. In case of needle stick or injury or other skin puncture/wound with spill, wash the wound thoroughly with soap and water. Encourage bleeding. Do not press/squeeze the wound.
4. Report immediately.

5. Maintain record.

10.3.3.2 Large Spills

Cover large spills (A spill that expands rapidly, presents an inhalation hazard, endangers people or environment >10 cm in size) using paper towels, cover it with 5000 parts per million (ppm) solution of sodium hypochlorite (1:10 dilution of 5.25% chlorine bleach to water or 10% Sodium hypochlorite solution) leave the area wet for 20 minutes , mop up while wearing PPE, and place them into the infectious waste trash can.

Small spill <10 cm in size use 1% Sodium hypochlorite solution and keep for 10 minutes. Prepare bleach solution fresh daily and keep it in a closed container because it degrades over time and in contact with the sun

10.3.3.3 Preparation of working solution of Hypochlorite

1% sodium hypochlorite is prepared by adding one part of commercially available 4% hypochlorite to 3 parts of water.

10.4 Avoidance of Contaminated Phlebotomy equipment

Tourniquets are a potential source of methicillin-resistant Staphylococcus aureus (MRSA), with up to 25% of tourniquets contaminated through lack of hand hygiene on the part of the phlebotomist or reuse of contaminated tourniquets. In addition, reusable finger-prick devices and related point-of-care testing devices (e.g. glucometers) contaminated with blood have been implicated in outbreaks of hepatitis B. To avoid contamination, any common-use items, such as glucometers, should be visibly clean before use on a patient, and single-use items should not be reused.

10.5 Sharps Safety Practices

- Be prepared
- Be aware
- Dispose with care
- Organize equipment at the point of use
- Make sure work space has adequate lighting
- Keep sharps pointed away from user
- Keep safety box within arm's length
- Assess patient's ability to cooperate
- Get help if necessary
- Ask patient to avoid sudden movement
- Maintain visual contact with sharps during use
- Be aware of staff nearby
- Control location of sharps to avoid injury to yourself and others

Figure 31: Improper handling of sharps

- Do not hand pass exposed sharps from one person to another
- Use predetermined neutral zone for placing / retrieving sharps
- Be accountable for sharps you use
- Check procedure trays, waste materials, and bedding for exposed sharps before handling
- Look for sharps/equipment left behind inadvertently
- Inspect safety box
- Always keep hands behind sharps
- Never put hands or fingers into sharps container
- If disposing sharps with attached tubing Be aware tubing attached to sharps can recoil and lead to injury Maintain control of both tubing and the device during disposal
- The person responsible for collecting garbage (non-sharp) may get exposed to possible sharps injury.
- It is important that proper segregation of waste is maintained in the laboratory.

Replace the safety box before they become overfilled

Overfilled safety box with protruding sharps could pose a risk of sharps injury during introduction of new sharps into it and even while closing it before disposal. Usually the safety boxes would have a fill line beyond which it should not be filled, suggested at the ¾ level. When a translucent safety box is being used the user can easily see the level up to which the box has been filled before deciding to replace the box with a new one. In case using a safety box that is not transparent, the user should check the fill level through the opening of the box with great care while maintaining a safe distance

Figure 32: Overfilled sharp container

10.6 Vaccination

Hepatitis B immunization should be offered to all those working in health-care facilities and especially to phlebotomists. One to two months after completing the three-dose series, the health worker should be tested to verify seroprotection (i.e. a concentration of antibodies to hepatitis B surface antigen of at least 10 milli-international units per milliliter [10 mIU/ml]). This is important because follow-up – including repeat serology testing after exposure to a patient positive for hepatitis B surface antigen – is unnecessary if the exposed person was known to have responded to the vaccine. Titres will decrease over time, even in those who are seroprotected, but the vaccinated person remains protected. In case of exposure, national guidelines on PEP for HBV exposure should be consulted or detailed instructions on the use of hepatitis B immune serum globulin (HBIG) and immunization against HBV are available from WHO. A fourth dose of hepatitis B vaccine should be offered to those who have completed their immunization but were tested 1–2 months after completing the vaccination and had a hepatitis

B surface antibody titre below 10 mIU/ml. If fewer than three doses of hepatitis B immunization have been given, a course of hepatitis B immunization should be provided or completed.

10.7 Post exposure prophylaxis (PEP)

Post exposure prophylaxis (PEP) is a medical response to prevent transmission of pathogens after potential exposure and refers to comprehensive management instituted to minimize the risk of infection following potential exposure to blood-borne pathogens (HIV, HBV and HCV). It includes first aid, counseling, risk assessment, relevant laboratory investigations based on the informed consent of the exposed person and source and depending on the risk assessment, the provision of short term (28 days) of antiretroviral drugs, along with follow-up evaluation.

One of the most dreaded hazards in healthcare facilities is the needle stick injury. Centre for Disease Control (CDC) and National AIDS Control Organization (NACO) recommend PEP for workers with needle stick injuries. PEP for HIV exposure is best when started within golden period of <2 hours and there is little benefit after 72 hours. The prophylaxis needs to be continued for 28 days.

See Annexure F for revised PEP guidelines by NACO (December 2014)

There is no recommended PEP for exposure to HCV. If feasible, testing of the source patient and health worker may be helpful to ensure workers compensation in the case that occupationally acquired infection is demonstrated. Research on PEP for HCV is ongoing to determine whether a regimen involving peginterfeon alfa-2b is effective.

Laboratory follow up for NSI is mandatory (NACO guidelines) as given in Annexure follow up to be done up to 6 months. (See Annexure F)

10.8 Biomedical Waste Management (See Next Section)

Chapter 11

Safe Disposal / Biomedical Waste Management

11.1 Learning Objectives

At the end of this chapter the learners should be able to understand:

a) The importance of BMW Management
b) The color coding system of segregation at source as per BMW 2016 rules
c) Mercury Waste Management
d) Transportation of wastes

11.2 Importance of Biomedical Waste Management

11.2.1. Categories of waste

BIO-MEDICAL WASTE (BMW) refers to any waste which is generated during the diagnosis, treatment or immunization of human beings/animals or in research activities.

It includes all samples past retention time, collection equipment, wastes from laboratory cultures, stocks or specimens of microorganisms live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishes and devices used for transfer of cultures, Waste sharps needles, syringes, scalpels, blades, glass, etc. that may cause puncture and cuts, discarded medicines and cytotoxic drugs, soiled waste (items contaminated with blood, and body fluids including cotton, dressings, soiled plaster casts, lines, beddings, other material contaminated with blood) and laboratory effluents. All these wastes should be disposed as per SCHEDULE 1 and SCHEDULE 2 (rule 5 and 6) BIO-MEDICAL WASTE (MANAGEMENT AND HANDLING) RULES, 2016 (under CPCB) under Ministry of Environment and Forest.

11.2.2 Purpose of Biomedical Waste Management

a) Protect people who handle waste items from accidental injury

b) Prevent the spread of infection to healthcare workers who handle the waste

c) Prevent the spread of infection to the local community

d) Safe disposal of hazardous materials to maintain safe environment

11.2.3 Reducing Risk of Infection from Medical Waste

a) Segregate waste at source

b) Use PPE when handling biomedical waste

c) Handle sharps with care

d) Do not fiddle with waste

e) Keep facility clean inside and out

f) Know steps for treating injuries (first aid)
Get fully immunized against tetanus and hepatitis B

g) In case of injury go for immediate evaluation to get post exposure prophylaxis

~80% is not harmful (Non-infectious)
~20% can be dangerous (Infectious)
~1% is sharps (Highly Infectious)

11.3 Biomedical Waste Segregation at source and color coding

cpcbenvis.nic.in/pdf/bmw_rules2016.pdf

Table: 11 Bio Medial Waste Management Guidelines 2016, Brief Overview

Color Coding	Type of waste	Pre treatment onsite	Treatment option as per schedule 1
Yellow	Human and animal anatomical waste, soiled waste like cotton, dressing and linen.		Incineration/ plasma pyrolysis
Yellow	Microbiological waste	Autoclaving	Incineration / plasma pyrolysis
	Liquid waste		As per schedule3
Red	Contaminated plastics (Recyclable)	Autoclaving	Autoclaving / Microwaving Recycle
White Translucent	Needle, syringe with fixed needle and other sharps	Autoclaving / Chemical disinfection	Autoclaving / Dry heat sterilization
Blue	Broken glassware	Chemical disinfection with sodium hypochlorite	Recycle
Green	Non infected waste		As per municipal solid waste management protocols

Note:

1. Color coding of waste categories with multiple treatment options as defined in Schedule I, shall be selected depending on treatment option chosen, which shall be as specified in Schedule I.
2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics.
3. Categories 8 and 10 (liquid) do not require containers/bags.
4. Category 3 if disinfected locally need not be put in

11.4 Mercury waste

1. All the mercury thermometers should be replaced by alcohol or digital thermometers.
2. In case the mercury thermometers are broken, contain the spilled mercury in a sealed container with the help of gloves and cardboard/paper.
3. The mercury can be returned back to the vendor supplying mercury thermometers.

11.5 Transportation of Biomedical Waste to Disposal Site

On site transport

- Wheel barrow
- Waste trolley
- Handcart

Offsite transport

- Dedicated waste collection vehicle
- Transport each waste category in separate bags
- If off site, obtain necessary license

Precautions during transport:

- Keep boxes upright
- Avoid direct contact of safety boxes with other waste or medical supplies in the same vehicle
- After transport, clean vehicle surfaces
- Keep safety boxes dry

PLEASE NOTE:
Also mandated are permission to generate waste (Form 1) and filing of the quantity of waste generated per year (Form 2) See Annexure I

Chapter 12

Monitoring and Evaluation in Pre-analytical Processes

12.1 Learning Objectives

At the end of this chapter learners should be able to understand:

a) The need for monitoring and evaluation in pre-analytical area
b) Procedure for developing quality indicators
c) Procedure for implementing Quality Indicators in pre-analytical processes

12.2 Need for a Monitoring System in pre-analytical area

A monitoring and evaluation system should be in place to offer surveillance for management of pre-analytical services and adverse events, and to plan quality objectives and the process of continual improvement and finally, to document improvements.

12.3 Quality indicators may include:

- Number and rate per month of sharps exposures and other occupational injuries occurring among health workers;
- Number and rate of patients per 100 patients with adverse events in response to phlebotomy such as hematoma, syncope, infection or nerve damage;
- Number of reported cases of blood borne pathogens transmitted during phlebotomy (disease surveillance for hepatitis B and C, and HIV) as part of a public health surveillance system that is capable of receiving and responding to reports of cases and clusters of infections;
- Number (and percentage) of phlebotomy sessions where essential equipment was not available and phlebotomy sessions were cancelled;
- Number (and percentage) of laboratory test results lost due to errors or poor quality; for example
  - Blood culture contamination rate;
  - Hemolysis and other sample rejection criteria;
  - Number of specimens with illegible or missing paperwork or labels;
  - Number of specimens that could not be processed due to inadequate sample volumes;
  - Number of samples rejected due to improper transportation
- Number (and percentage) of trained staff in the health-care facility working in phlebotomy;
- Number (and proportion) of juniors who are supervised by trained staff.

12.4 Procedure:

- The collection and supervisory staff are encouraged to fill in the NCE/incidents register (Annexure A) on a daily basis
- These should be compiled and presented at Management Review Meeting
- The details should include the type and detail of incident, immediate action, corrective and preventive actions, root cause analysis and the time taken to rectify the problem
- At the end of the month, a collated summary may be entered into Labs For Life Monitoring and evaluation module; (Annexure H)
- Trends in pre-analytical activities can be analyzed further for remedial action, setting of quality objectives and continual improvements.

  Monitoring of NCE can be done on the Labs For Life Monitoring tool at "http://labsforlife.in/Default.aspx"

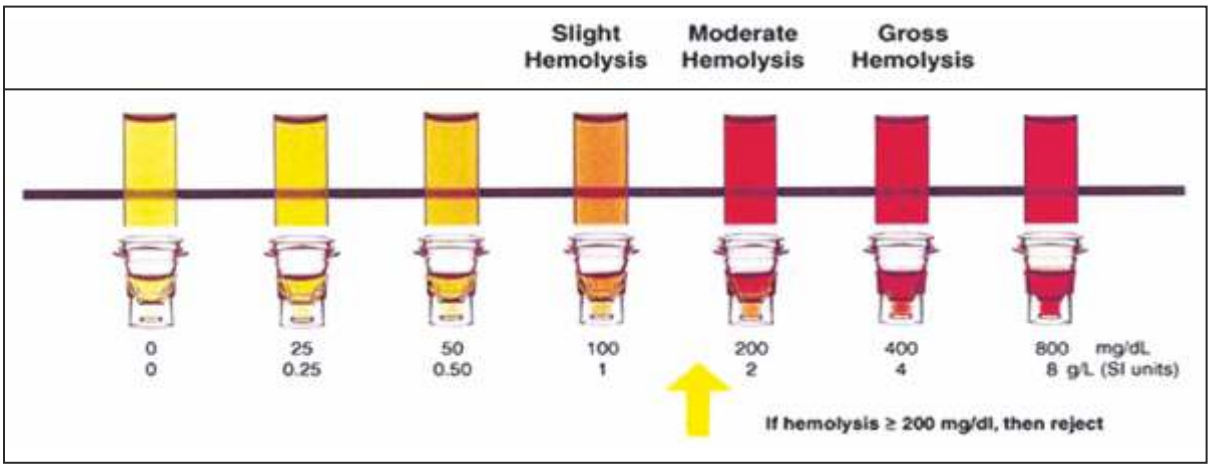


Figure 33: Hemolysis Grading

Example : **% of sample rejected due to hemolysis**

$$= \frac{\text{No. of 200 mg/dl and above hemolysis in a month}}{\text{Total no. of samples in that month}} \times 100$$

References

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9. NABL 112, Specific Criteria for Accreditation of Medical Laboratories
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11. RNTCP Guidelines 2013
12. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4e (TIETZ TEXTBOOK OF CLINICAL CHEMISTRY (BURTIS)) 4th Edition
13. WHO guidelines on drawing blood: best practices in phlebotomy

Abbreviations

ACD	:	Acid-Citrate-Dextrose
ACTH	:	Adreno-Corticotropic Hormone
ADA	:	American Diabetic Association
ALT	:	Alanine Transaminase
APTT	:	Activated Partial Thromboplastin Time
AST	:	Aspartate Aminotransferase
BMW	:	Biomedical Waste Management
CBC	:	Complete Blood Count
CDC	:	Centers For Disease Control And Prevention
CLSI	:	Clinical and Laboratory Standards Institute
CPCB	:	Central Pollution Control Board
CVP	:	Central Venous Pressure
DBS	:	Dried Blood Spot
DNA	:	Deoxyribonucleic Acid
EDTA	:	Ethylene Diamine Tetra Acetic Acid
ESR	:	Erythrocyte Sedimentation Rate
FNA	:	Fine Needle Aspiration
HCL	:	Hydrochloric Acid
HCT	:	Hematocrit
HCW	:	Health Care Worker
HIV	:	Human Immunodeficiency Virus
HPV	:	Human Papilloma Virus
IBBS-HIV	:	Integrated Biological And Behavioral Surveillance
ISO	:	International Organization For Standardization
IUD	:	Intra-Uterine Device
K+	:	Potassium Ion
LBC	:	Liquid Based Cytology
LDH	:	Lactate Dehydrogenase
LIS	:	Laboratory Information System
NABL	:	National Accreditation Board For Testing And Calibration Laboratories
NAOH	:	Sodium Hydroxide
NCCLS	:	National Committee For Clinical Laboratory Standards

NSI	:	Needle-Stick Injury
PCR	:	Polymerase Chain Reaction
PEP	:	Post Exposure Prophylaxis
PO-4	:	Phosphate Ion
PPE	:	Personal Protective Equipment
RBC	:	Red Blood Cell
RCF	:	Relative Centrifugal Force
RNTCP	:	Revised National Tuberculosis Control Program
RPM	:	Revolutions Per Minute
SOP	:	Standard Operating Procedure
SST	:	Serum Separation Tubes
TRF	:	Test Requisition Forms
WHO	:	World Health Organization

GLOSSARY

Accessioning	An ordered test or group of tests on a particular specimen which has been formally received by a lab or other health care service and has received an accession number.
Anticoagulants	Substances having the effect of retarding or inhibiting the coagulation of the blood
Arteriospasm	Spasm of an artery, resulting in a decrease of its caliber.
Bio- Hazards	A risk to human health or the environment arising from biological work, especially with microorganisms.
Biomedical Waste	Solid waste generated during the diagnosis, testing, treatment, research or production of biological products for humans or animals. Biomedical waste includes syringes, live vaccines, laboratory samples, body parts, bodily fluids and waste, sharp needles, cultures and lancets.
Directory of Services	List of services offered by the lab which includes the test specification, as per ISO:15189 clause 5.4
Dysuria	Painful or difficult urination.
Epidemic	A widespread occurrence of an infectious disease in a community at a particular time.
Frozen Sections	The frozen section procedure is a pathological laboratory procedure to perform rapid microscopic analysis of a specimen.
Healthcare Professional	An individual who provides preventive, curative, promotional or rehabilitative health care services in a systematic way to people, families or communities.
Hypercoagulability	A condition in which there is an abnormally increased tendency toward blood clotting (coagulation).
Laboratory Information System	A software-based laboratory and information management system with features that support a modern laboratory's operations.
Monitoring & Evaluation	A process that helps improving performance and achieving results. Its goal is to improve current and future management of outputs, outcomes and impact.
Non-Conforming Event/ Adverse Event/ Incidence	An untoward incident, therapeutic misadventure, iatrogenic injury, or other adverse incident directly associated with care, or services provided within the jurisdiction of a medical center, outpatient clinic or other healthcare facility.
Osteomyelitis	An infectious usually painful inflammatory disease of bone.
Petechiae	A small red or purple spot caused by bleeding into the skin
Phlebotomy	The removal of blood from a vein, usually with a needle and syringe or other container, for diagnostic or therapeutic purposes.

Pre-analytical Processes	Describing any variable whose value can affect the outcome of a subsequent analysis
Pulse Field Gel Electrophoresis	A technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.
Quality	The standard of something as measured against other things of a similar kind; the degree of excellence of something.
Quality Assurance	The maintenance of a desired level of quality in a service or product, especially by means of attention to every stage of the process of delivery or production.
Quality Indicators	Measures that can be used to monitor and evaluate the quality of important governance, management, clinical, and support functions that affect patient outcomes
Standard Precautions	The basic level of infection control that should be used in the care of all patients all of the time to reduce the risk of transmission of microorganisms from both recognized and non-recognized sources of infection.
Surveillance	Close observation or supervision maintained over a person, group, etc.
Timed Collection	The collection of a specimen, such as a urine or stool sample, for a specific period of time.
Training of Trainers	TOT is for teaching/training personnel practising as professionals in a given field who accompany trainees in their work environment. It covers a wide range of skills: knowledge specific to the field in question (general, technical or scientific) It also includes educational, psychological and sociological skills; management skills; familiarity with the world of work; and knowledge of training schemes and target audience. Lastly it also covers training related to course design, organisation and implementation as well as the content of training activities, i.e. imparting knowledge, know-how and skills.

ANNEXURES

Laboratory requisition Form - Annexure A1

To be filled in by the Clinician

Name of the Laboratory: ABC	**Department: XYZ**

Patient's Name: Sex: M/F Date:

Age DOB (Yrs /month): Ward/ Department:

Name and signature of Clinician requesting the tests:

Contact Address and Telephone numberof referring doctor:

Address and Telephone Number of patient:

Examination Requested For: (May give Menu)	Clinical Information

To be filled in by the lab; registration

Tracking (Permanent) Record Number:		Laboratory ID:	
Date and Time of Sample Collection:			
Date and Time of Sample Receipt (Samples from outside)			

To be filled in by technician

No: of attempts	Tubes/ Containers/ Samples Collected/ Received												
	Culture	Coag	Plain	Heparin	EDTA	ACD	Fluoride	ESR	Urine	Stool	Swabs	Histo/ Cyt	Others

Name and Signature of Technician

NCE Reporting Form - Annexure A2

Name of Hospital/ Logo	Document identity	Page 1 of….
Name of document		
Prepared by	Original date of issue:	
Issued by	Revision Date:	
Edition Number:	Next Review date:	

NCE Reporting format: Sample

S. No/ Date/Reported by	
Type of Incident**	
Immediate Action	
Corrective Action	
Preventive Action	
Root Cause Analysis	
Time taken for resolution	

Signature of QM

Sample format Informed Consent - Annexure A3

Name of Hospital/ Logo	Document identity	Page 1 of….
Name of document		
Prepared by	Original date of issue:	
Issued by	Revision Date:	
Edition Number:	Next Review date:	

Sample format: Informed Consent

- Fine needle aspiration cytology is a diagnostic procedure used to investigate superficial lumps or masses where a small needle is inserted to draw a sample of tissue from the lump or mass.
- A needle aspiration biopsy is safer and less traumatic than an open surgical biopsy and significant complications are usually rare. Common complications include bruising and soreness.
- If you have any bleeding disorders/ are on aspirin or non-steroidal anti-inflammatory medications / anticoagulants, please indicate. It is ideal to stop anticoagulation medication for at least 3 days before the test.
- Any other significant medical condition may also be indicated on the consent form.
- Since the biopsy is very small, it is possible that the problematic cells will be missed resulting in a false negative result.
- There is also a possibility that the tissue taken will not enable a definitive diagnosis.
- Clinical and radiological correlation is required in all cases.
- In the case of small/deep seated lesions, Ultrasound guidance may be required.
- If sample is insufficient on one puncture, repeat sampling may be required.
- After the procedure, mild analgesics may be used to control pain. Since sterility is maintained throughout the procedure, infection is rare. But should an infection occur, it should be treated with antibiotics.
- Slight bleeding may also happen
- You may clarify any further query with the Pathologist about procedure. A slight bruise may also appear. Other complications depend upon the body part on which the biopsy takes place

P.T.O

Informed Consent - Annexure A3

Name of Hospital/ Logo	Document identity	Page 1 of….
Name of document		
Prepared by	Original date of issue:	
Issued by	Revision Date:	
Edition Number:	Next Review date:	

Consent

Name …….

Address ……

Date ……..

Any history of bleeding disorders / hypertension / vaso-vagal attacks (fainting)……

Any History of aspirin / anticoagulants …….....

I have read the above information in a language which I understand.

I understand the information that has been given to me about FNAC

I have been given adequate opportunity to ask questions to obtain further information.

All of my questions have been answered to my satisfaction.

I have no further questions.

I give my permission to perform FNAC

Signature (of the patient or guardian in the case of children)

Field Sample Collection Form - Annexure A4

CASE ID NUMBER_____

Case Referral Form (during outbreaks)

SPECIMEN INFORMATION

Specimen source/type:
Fresh stool/Stool swab/Blood/Serum/Blood culture/CSF/
Throat swab/nasopharyngeal swabs / Sputum / Urine/
others (please list)_____

Collection date____/____/_____
DD MM YYYY
Time collected_____/_____
HH MN

INFORMATION ABOUT PERSON/FACILITY REQUESTING TEST

Name & designation of official referring the specimen: _____

Phone Number_____ Email_____

In case specimen being sent from the field: _____
Village Block District

In case specimen being sent from facility (PHC / CHC / District hospital), name of facility:_____

PATIENT DEMOGRAPHICS

Name_____ Age _____/_____ Sex ☐ Male ☐ Female
Years Months

S/O, D/O,W/O _____

Address _____ _____ _____ _____ _____
Street Address Village Block District State

Patient Phone Number: _____

PATIENT CLINICAL HISTORY

Date of Onset of Illness_____/_____/_____
DD MM YYYY

Time of Onset of Illness _____ ☐AM ☐PM

Suspected Syndrome: Acute Diarrhea/Acute Haemorrhagic fever/Acute Jaundice/Acute Neurological/ Acute Respiratory/others (name) _____

_____

Clinical Signs/Symptoms (TICK ALL THAT APPLY):

Fever / Haemorhagic rash / Other haemorhagic manifestations (name)_____ /Jaundice/ Acute onset Cough/Diarrhea/ Nausea & Vomiting/Blood in Stool/Deterioration of mental functions/acute paralysis/convulsions/ Other (please specify) _____

_____

Presumptive Diagnosis:_____

Antimicrobial Treatment Received Prior to Specimen Collection: ☐Yes ☐No ☐Unknown

If Yes, Specify which antibiotic: _____

Hospitalized for this illness: ☐Yes ☐No ☐Unknown

Laboratory Examination Requested

Facility to which specimen is being sent: _____

Type of Examination Requested (Tick all that apply):

Microscopy/ Bacterial Culture/ Antimicrobial Susceptibility Testing/Antigen or Antibody detection for_____ /Others (name)_____

Patient Feedback Form - Annexure A5

Name (Optional):	Date:
Age: Sex:	

Are you filling in this questionnaire for:

○ Your Friend ○ Yourself ◉ Your Relative

How long did you wait before you were greeted by lab staff?

○ 15 - 20 minutes ○ 20 - 30 minutes ○ Longer than 30 minutes

How long did you wait before your procedure was started?

○ 15-20 minutes ○ 20- 30 minutes ◉ Longer than 30 minutes

Please give the answer

S.No	Question	Yes	No
1	Were there sufficient signs on the building?		
2	Is the laboratory located within a convenient area?		
3	Were you greeted in a friendly and polite manner?		
4	Did the lab employee confirm bleeding had stopped before releasing you?		
5	Did the lab employee label your samples in front of you?		
6	Did they inform you about the reporting time?		

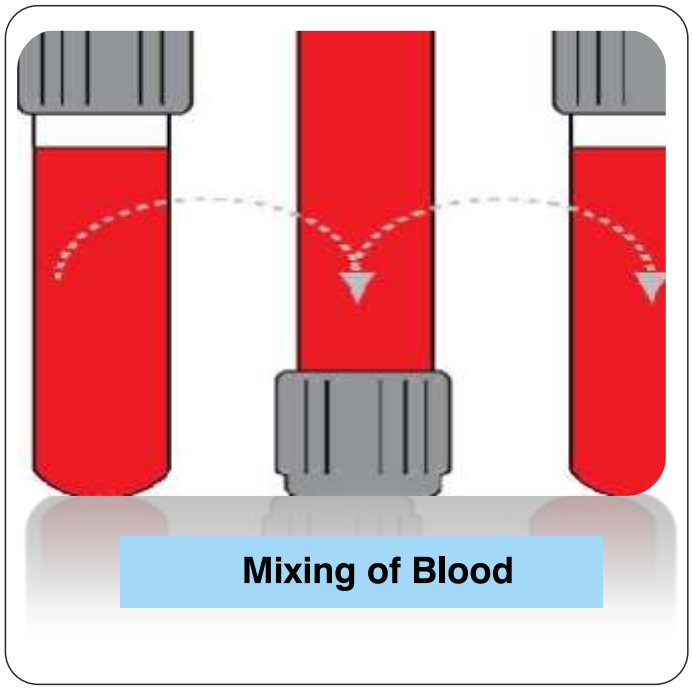
S.No	Question	Yes	No
1	How would you rate staff clinically experienced and knowledgeable?		
2	How would you rate patient privacy in the labs		
3	Was this area clean and prepared for your visit or needs?		
4	Was the blood samples collected in a skillful manner?		
5	Please rate your overall experience		

Please tell us who or what had a positive impact on your visit.

Please suggest one or more area(s) of improvement that would have made a significant difference in your overall experience.

Order of Specimen Draw - Annexure B

Closure	Collection Tube	Mix by Inverting
	Blood Culture	------
	Citrate Tube	3 to 4 times
	Gel Separator Tube/ Serum Tube (Plastic/glass)	5 times (plastic) none (glass)
	Heparin Tube	8 to 10 times
	EDTA Tube	8 to 10 times
	ACD tube	8 to 10 times
	Fluoride Tube	8 to 10 times
	ESR Tube	8 to 10 times

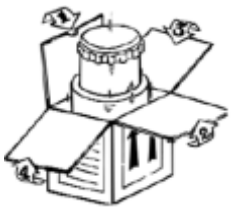


Mixing of Blood

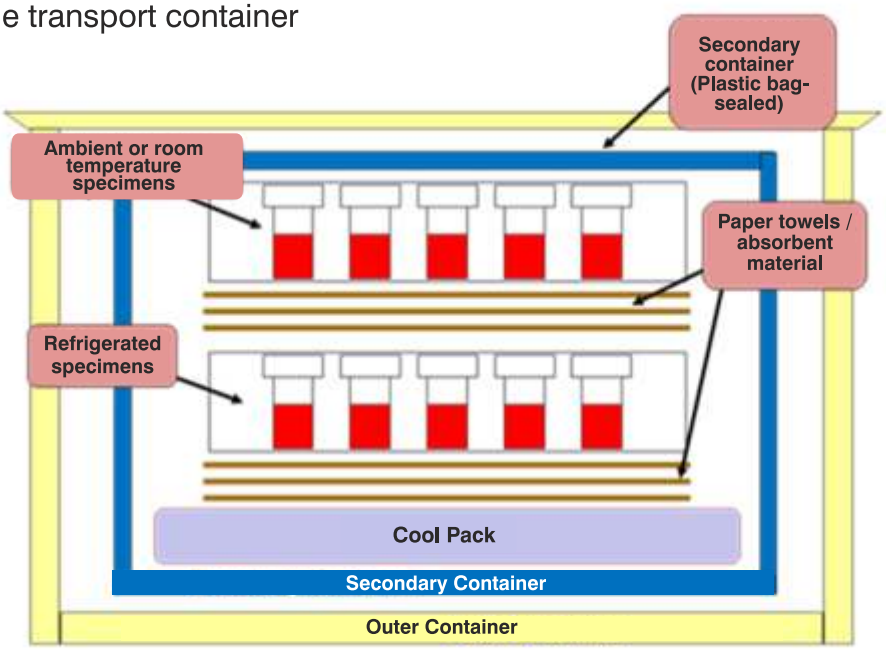
Note: Handle all biologic samples and blood collection "sharps" (lancets, needles, luer -adapters and blood collection sets) according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury) since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in used needle protector if the blood collection device provides one. It is not recommend shielding used needles, but the policies and procedures of your facility may differ and must always be followed. Discard any blood collection "sharps" in biohazard containers approved for their disposal.

Sample Packaging Guidelines - Annexure C

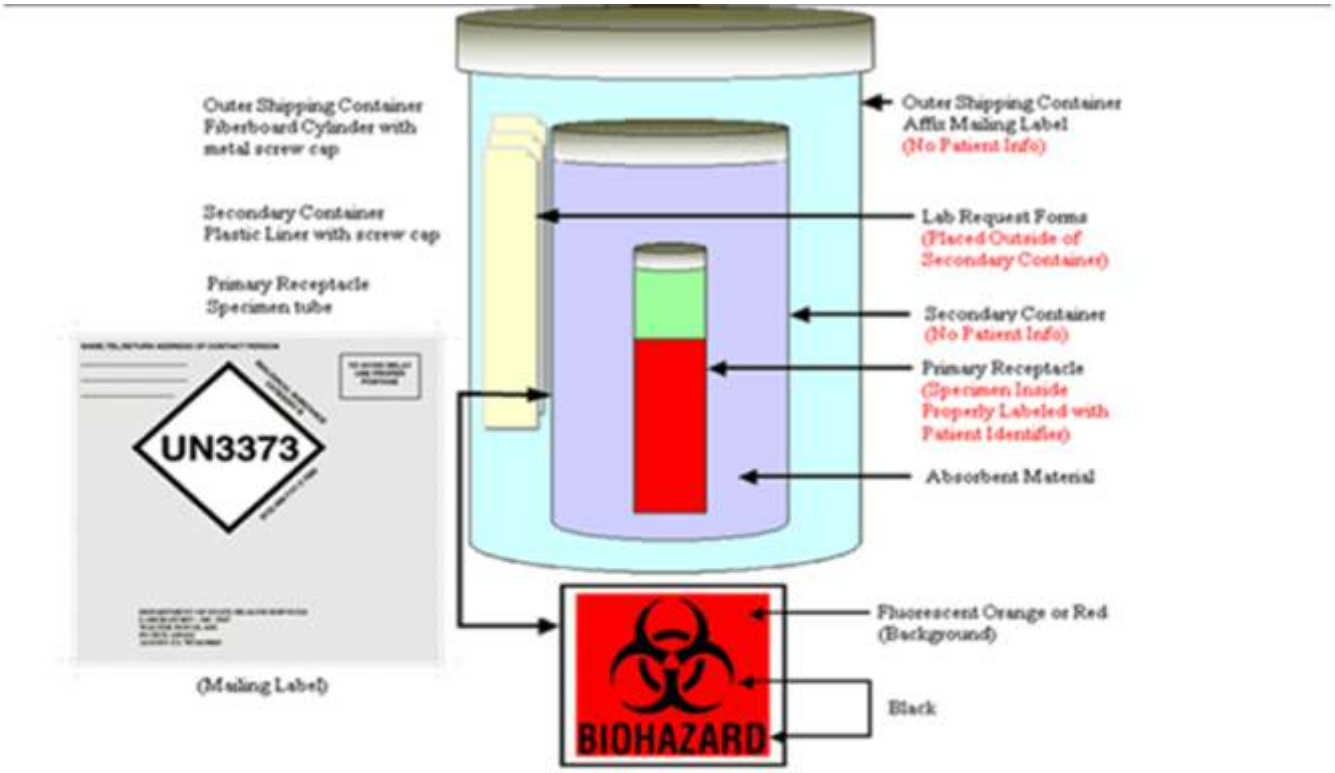
Schematic illustration of Packaging and transporting the specimens

Step No	Diagram	Activity
1		Packing the sample with absorbent
2		Packing in a primary container
3		Sample should be packed in air and watertight container
4		Pack the Primary container in secondary one.
5		Pack the content in cardboard, wood

Cross section of the transport container

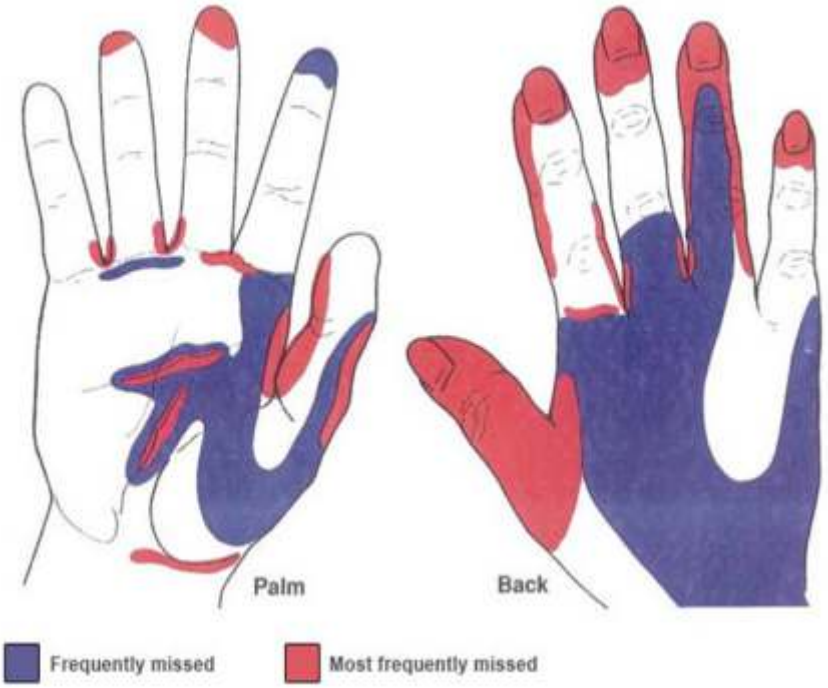


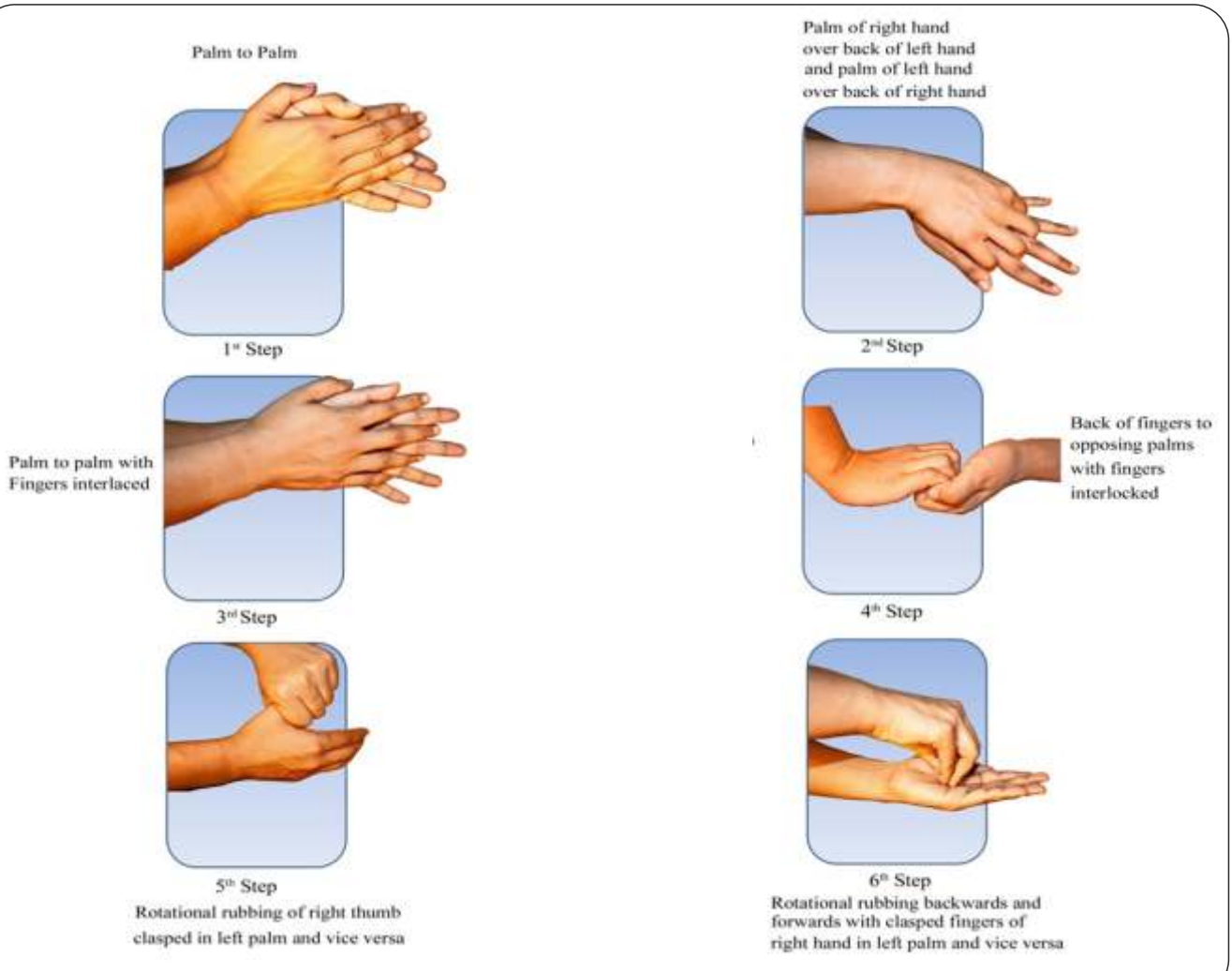
DO NOT put any patient information on outer container or secondary containers or lids.



Biohazard Label should be on Secondary Container.
DO NOT put Biohazard Label on Outer Container.

Hand Hygine - Annexure D





How to Handwash?

WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB

Duration of the handwash (steps 2-7): 15-20 seconds

Duration of the entire procedure: 40-60 seconds

0

Wet hands with water;

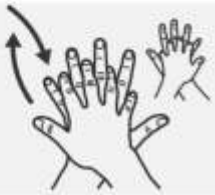
1

Apply enough soap to cover all hand surfaces;

2

Rub hands palm to palm;

3



Right palm over left dorsum with interlaced fingers and vice versa;

4

Palm to palm with fingers interlaced;

5

Backs of fingers to opposing palms with fingers interlocked;

6

Rotational rubbing of left thumb clasped in right palm and vice versa;

7

Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8

Rinse hands with water;

9

Dry hands thoroughly with a single use towel;

10

Use towel to turn off faucet;

11

Your hands are now safe.

World Health Organization | Patient Safety — A World Alliance for Safer Health Care | SAVE LIVES Clean Your Hands

May 2009

Color Code of Needle - Annexure E

Size		Color		Length					
OD(mm)	Gauge	Color & Code		1/2"	5/8"	3/4"	1"	1 1/4"	1 1/2"
0.30	30G		Light yellow						
0.33	29G		Red						
0.36	28G		Blue-green						
0.40	27G		Grey						
0.45	26G		Brown						
0.50	25G		Orange						
0.55	24G		Purple						
0.60	23G		Blue						
0.70	22G		Black						
0.80	21G		Green						
0.90	20G		Yellow						
1.10	19G		Beige						
1.20	18G		Pink						
1.60	16G		white						

Published Guidelines Post Exposure Prophylaxis (NACO)
Laboratory Follow-up guidelines (NACO)
- Annexure F

No. T-11020/52/2007-NACO (ART)
Government of India
Ministry of Health and Family Welfare
National AIDS Control Organisation

Office Memorandum

Subject: Revised Guidelines for Post Exposure Prophylaxis (PEP) for HIV.

In supersession to the existing guidelines on Post Exposure Prophylaxis (PEP), it has been decided to revise the guidelines regarding recommendations and regimen used for Post Exposure Prophylaxis.

The revised guidelines are as follows

1. PEP recommendations

a. Occupational Exposure

Exposure Codes *	HIV Source Code**	PEP Recommendations	Duration
1	1	Not warranted	
1	2	Recommended	
2	1		
2	2		
3	1		
2/3	Unknown	Consider PEP, if HIV prevalence is high in the given population & risk categorisation	

* - Details of Exposure codes at Annexure 1

** - Details of HIV Source Code at Annexure 2

b. **In case of Sexual Assault:** PEP should be provided to exposed person in case of sexual assault as a part of overall package of post sexual assault care.

2. PEP regimen

a. Wherever PEP is indicated and source is ART naive or unknown: **recommended regimen is Tenofovir 300 mg + Lamivudine 300 mg + Efavirenz 600 mg once daily for 28 days.** Wherever available, single pill containing these formulations should be used. Dual drug regimen should not be used any longer in any situation for PEP. b. The first dose of PEP regular should be administered as soon as possible, preferably within 2 hours of exposure and the subsequently dose should be given at bed time with clear instruction to take it 2-3 hours after dinner & to avoid fatty food in dinner. c. In case of intolerance to Efavirenz, regimen containing Tenofovir + Lamivudine + PI (ATV/r or LPV/r) can be used after expert consultation by an experienced physician.

d. In case of exposure where source is on ART, Tenofovir 300 mg + Lamivudine 300 mg + Efavirenz 600 mg should be started immediately. And an expert opinion should be sought urgently by phone/e-mail from CoE/ART Plus centre.

e. Appropriate and adequate counselling must be provided regarding possible side effects, adherence and follow up protocol.

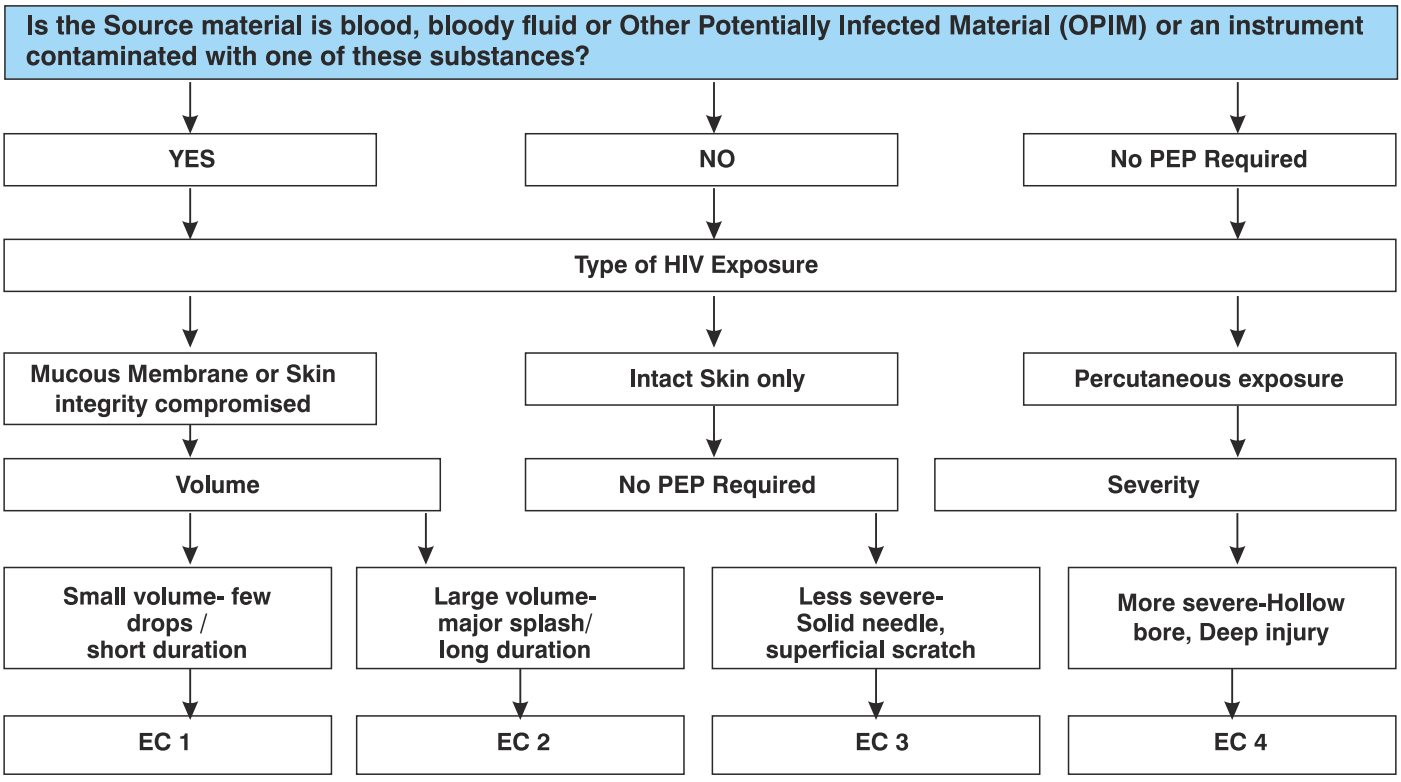
Dr A.S. Rathore
DDG,CST

To.
The Project Director, All SACS

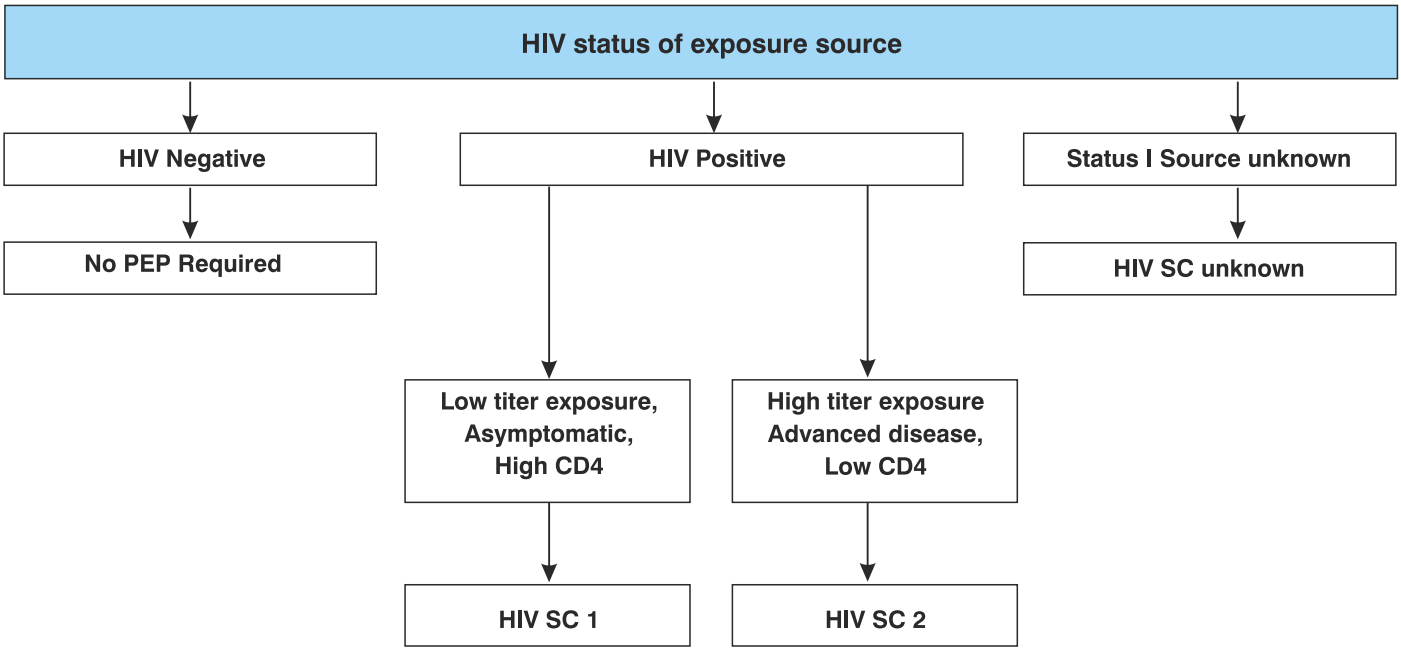
Copy to:
JD(CST)/ Officer In Charge (CST)
All Regional Coordinators
Nodal Officer, All ART Centres
Programme Directors of CoE / PCoE

Copy for Information to:
PPS to AS, MoHFW
PS to JS, NACO
DS (A&P)

Annexure 1: HIV Exposure Code



Annexure 2: HIV Source Code



Laboratory Investigations For Occupational Exposure

Timing	In Persons Taking PEP (standard Regimen)	In Persons Not Taking PEP
Baseline (within 8 days after AEB)	HIV, HCV, anti-HBs, CBC, Transaminases	HIV, HCV, anti-HBs
Week 2 and 4	Transaminases* Complete blood count**	Clinical monitoring for Hepatitis
Week 6	HIV-Ab	HIV-Ab
Month 3	HIV-Ab, anti-HCV, HBsAg, Transaminases*	HIV-Ab, anti-HCV, HBsAg
Month 6	HIV-Ab, anti-HCV, HBsAg, Transaminases*	HIV-Ab, anti-HCV, HBsAg

* Transaminases should be checked at week 2 and 4 to detect hepatitis in case the exposed personcontracted HBV from the AEB.

** For persons started on AZT-containing PEP regimens.

Checklist for Auditing, (as per clause 5.4 and 5.7 ISO 15129:2012) - Annexure G

5.4 Pre-examination processes				
5.4	**Pre-examination processes**	**What to look for**	**Y/P/N**	**TA Provided**
		5.4.1 General		
5.4.1 General	Does the laboratory have a documented **procedures** and information for pre-examination activities to ensure the validity of the results of examinations?	**Path of Sample flow** Follow a patient specimen beginning with test ordering through patient identification, phlebotomy/collection, labelling, transport, receipt and processing, delivery to test area, analysis, result review, and reporting. Determine if practice matches related policies and procedures.		
		5.4.2 Information for patients and users		
5.4.2 Information for patients and users	Does the lab provide information for users which includes a list of examinations offered, instructions for collection, transport, handling of samples and specimens and relevant performance specifications of the tests	Request forms have space for the inclusion of, but not limited to: 5.4.3 'a' through 'g' Look for displays of timings of hours of work, TAT, brochures or pamphlets for patient collected activities, DOS, consent form , signage for utilities and services, display of cashless services, Complaint redresses policy. All policies should be available in local languages.		
		5.4.3 Request form information		
5.4.3 Request form information	Does the request form contains sufficient information to identify the patient and authorised requester and provides pertinent clinical data? Are written polices available concerning verbal requests for sample examinations?	Request forms have space for the inclusion of, but not limited to: 5.4.3 'a' through 'g' ID of patient and requester; type of primary sample; examinations requested; relevant clinical information; date and time of primary sample collection and receipt. Policy for management of verbal requests.		
		5.4.4 Primary sample collection & handling		
5.4.4.1 (General)	Primary sample collection manual	Availability of primary sample collection manual at all collection points including wards, labour room, OT, ICUs etc.		

Checklist for Auditing, (as per clause 5.4 and 5.7 ISO 15129:2012) - Annexure G

5.4 Pre-examination processes				
5.4	**Pre-examination processes**	**What to look for**	**Y/P/N**	**TA Provided**
5.4.4.2 Instructions for pre-collection activities	Are the instructions provided under 5.4.2 also present in the sample collection manual under 5.4.4.2 and are being followed? Is help being provided to the patients and users in understanding and compliance.	User manual containing the information as detailed in Clause 5.4.4.2 'a' to 'e'.		
5.4.4.3 Instructions for collection activities	Does the lab provide adequate instructions for collection activities 1. Preparation of the patient 2. Type of collection container and amount of specimen to be collected 3. Need for special timing for collection 4. Types and amounts of anticoagulants 5. Need for special handling between time of collection and time received by the laboratory (e.g. refrigeration, immediate delivery) 6. Proper specimen labelling 7. Need for appropriate clinical data, when indicated	Written collection procedure defining criteria for patient identification (5.4.4.3 'a' through 'h'). Training log on sample collection (see annexure sample logging format) All primary specimen containers must be labelled with 3 identifiers at the time of collection. Phlebotomy supplies such as blood collection tubes and collection devices are used within their expiration date and stored per manufacturer's instructions. Personnel confirm the patient's identity by checking at least two identifiers before collecting a specimen.		
5.4.5 Sample transportation				
5.4.5 Sample transportation	Does the laboratory's instructions/procedure for post-collection activities include packaging and labelling to indicate the general nature of the materials transported? Does the laboratory monitor how the samples are transported to the laboratory &ensure the integrity of samples and safety of the carrier and environment? Does the laboratory package and ship infectious material in accordance with applicable national & international regulations? Transport personnel are trained in appropriate safety and packaging procedures Is there a documented tracking system to ensure that all specimens are actually received	User manual, records of monitoring the time, temperature and safety conditions for samples transported to the lab in accordance with Clause 5.4.5 'a' to 'c' Specimens are packaged appropriatelyto prevent contamination of workers, patients, or the environment and transported within acceptable timeframes. Look at the log pre transportation preparation, look for aliquot tubes in the stock, gel packs in freezer, centrifuge maintenance etc Records of review of applicable regulations. Records of training for all personnel involved in transport of specimens Specimen shipping/transport logs andrecords of follow up for specimens not received.		

Checklist for Auditing, (as per clause 5.4 and 5.7 ISO 15129:2012) - Annexure G

5.4 Pre-examination processes				
5.4	**Pre-examination processes**	**What to look for**	**Y/P/N**	**TA Provided**
5.4.6 Sample reception	Is there is a system to positively identify all patient specimens, specimen types, and aliquots at all times? Has the laboratory maintained an accession log of all samples received and developed criteria for acceptance and rejection of primary samples? Is the date and time, that the specimen was received by the laboratory recorded? Has test requisition data elements been entered accurately into the laboratory information or record system?	5.4.6 a-f Each specimen container identifies the patient uniquely. This may be text-based, numeric, bar-coded, etc. Check for traceability of primary samples and sample portions or aliquots to identified individuals from whom the specimens were taken. Review accession register/log. The laboratory has an ongoing mechanism to ensure the accuracy of manual entries.		
5.4.7 Pre-examination handling, preparation and storage	Are the operating speeds of centrifuges checked at least annually as needed for the intended use and that this is done in a safe manner. Are the Refrigerator/freezer temperatures checked and recorded daily? Does the laboratory have a documented procedure for processing and reporting all samples marked as urgent? Does the laboratory ensure that after results have been reported the samples are stored for a specified time under conditions that ensure their stability so that if required the examination may be repeated? Does the procedure specify time limits for requesting additional or further examinations on the same primary sample? Is there a reagent logbook for lot number and dates of opening that reflects verification of new lots? Are testing personnel identified on the requisition and record?	Records of verification of operating speeds of centrifuge documented at least annually. The laboratory has defined acceptable temperature ranges for these units. If temperature(s) are found to be outside acceptable range, laboratory documents appropriate corrective action, which includes evaluation of contents for adverse effects. The identity of the individual recording the temperature(s) is documented (recording the initials of the individual is adequate). Procedure for handling samples marked as urgent that include the required details as listed. Sample storage and retention policy & records. Samples are stored under appropriate conditions to maintain the stability and when no longer required are disposed of in a safe manner, according to biosafety regulations. The person who performed the procedure must be identified on the report for purposes of audit trail.		
5.7 Post analytical				
5.7.2 Storage, retention an disposal of clinical samples	Lab should have a documented procedure for identification, collection, indexing, access, storage, maintenance and safe disposal of clinical samples	See procedures being carried out. Look at QSP to see if NABL 112 guidelines are followed See the Bio-medical waste management. Ensure 1998 CPCB rules are followed		

For a detailed BMW checklist please refer to the Kayakalp checklist of NHM

Preanalytical Module - Annexure H

Total number of samples received in the reporting month.

No. of incidents in sample collection

Mislabel/Lack of three identifiers (name, ID no., age/sex), wrong identification of sample/patient, transcriptional error, leakage, blood-stained form, needle stick injuiry, splash/spill of blood)

Key reasons for incidents in sample collection

(multiple reasons may exist)

- Mislabel / lack of three identifiers (name, ID no., age/sex)
- Wrong identification of sample/patient
- Transcriptional error
- Leakage
- Blood-stained form
- Needle stick injury
- Splash/spill of blood

No. of samples rejected

Sample rejection criteria: Insufficient quantity, inappropriate/incorrect container, inappropriate temperature, inappropriate transportation, Haemolysis, clotted EDTA samples, Lipemic sample, inappropriate/incorrect form, leakage of sample, inadequate / incorrect preservative etc.

Key reasons for sample rejection

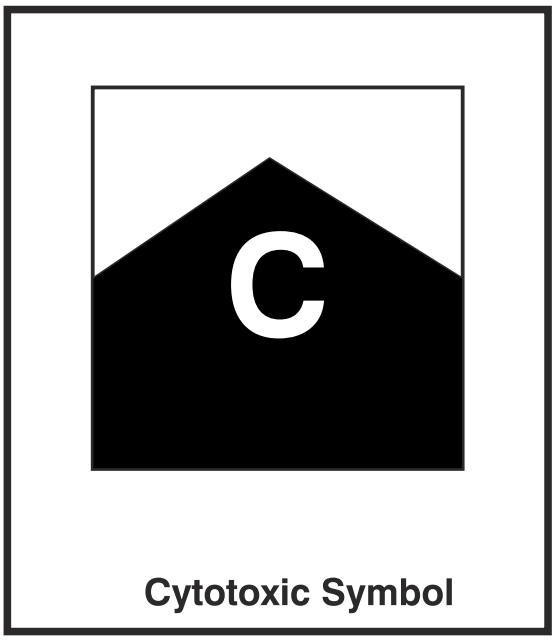
(Multiple reasons may exist)

- Insufficient quantity
- Inappropriate/incorrect container
- Inappropriate temperature
- Inappropriate transportation
- Haemolysis
- Clotted EDTA samples
- Lipemic sample
- Inappropriate/incorrect form
- Leakage of sample
- Inadequate / incorrect preservative

No. of complaints received in sample collection

LABEL FOR BIO-MEDICAL WASTE CONTAINERS/BAGS

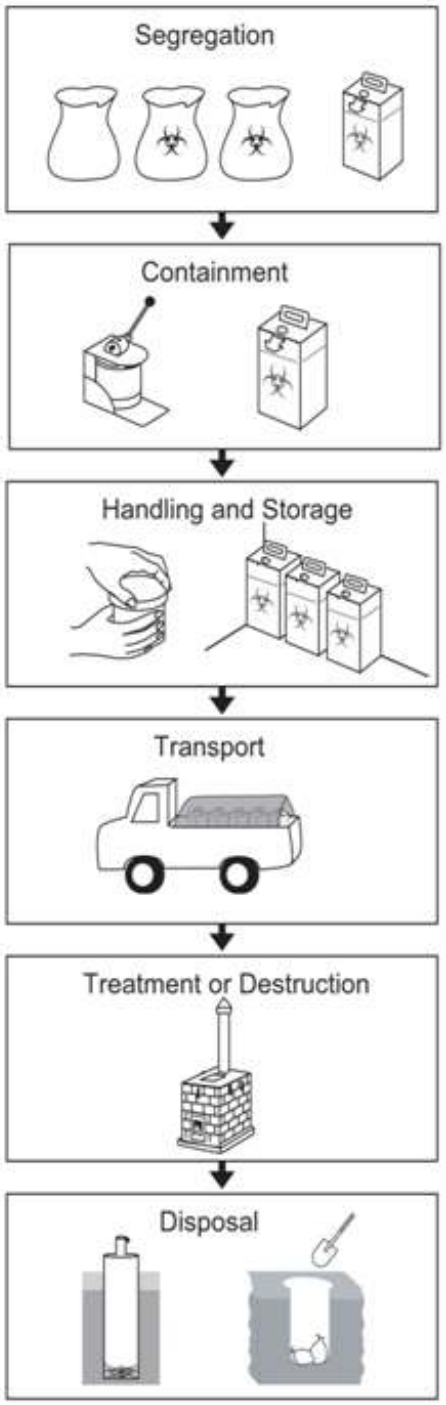
Biohazard Symbol



Cytotoxic Symbol

HANDLE WITH CARENote: Label shall be non-washable and prominently visible.

Key Steps in Waste Management



Segregation

Containment

Handling and Storage

Transport

Treatment or Destruction

Disposal

Laboratory Quiz - Annexure J

I. Phlebotomy Equipment

1. Under standard precautions, what personal protection equipment must the phlebotomist use for every blood collection procedure?
    a. Gloves
    b. Gown
    c. Gown, gloves, sharps and safety box
    d. Sharps and safety box
2. Which one of the following is the purpose for using a tourniquet:
    a. To allow good flow of blood
    b. To ensure patient feels less pain
    c. To make veins easier to locate and feel
    d. To stop arterial and venous flow of blood
3. Which one of the following is the correct concentration of alcohol used for skin cleansing?
    a. 100% Alcohol
    b. 95% Alcohol
    c. 70% Alcohol
    d. 50% Alcohol
4. After applying alcohol to the puncture site it should be dried by:
    a. Wiping with a clean gauze
    b. Blowing air on the site
    c. Letting it dry on its own
    d. No need to let it dry

II. Phlebotomy technique

1. Which of the following is the correct way to establish patient identification?
    a. Ask the patient “Are you Mr. Gerald?”
    b. Verify patient identification with a visitor
    c. Ask the patient his / her all names and verify this with the requisition and wrist band or medical record if available
    d. Ask the nurse
2. Which of the following should NEVER be the same on any two patients?
    a. Date of birth
    b. First names
    c. Last names
    d. Medical record number
3. Recommended duration for tourniquet application is:
    a. Less than 1 minute
    b. 2 minutes
    c. 2 to 3 minutes
    d. Till the time required quantity of blood has been drawn

4. During blood collection, when should the tourniquet be released?
    a. After the desired volume of blood has been drawn
    b. Once blood starts flowing into the first tube / syringe
    c. After 10mL of blood has been drawn
    d. After the needle has been drawn from the vein

5. Which one of the following sequence of preference is recommended for vein selection during venous blood collection?
    a. Cephalic → Basilic → Median cubital
    b. Median cubital → Basilic → Cephalic
    c. Basilic → Cephalic → Median cubital
    d. Median cubital → Cephalic → Basilic

6. Which of the following correctly indicates inappropriate blood collection site(s)?
    a. Site with hematoma
    b. Edematous site
    c. Arm on side of mastectomy
    d. Site with tattoos
    e. All of the above

7. Which one of the following is the correct site cleansing procedure during phlebotomy?
    a. Rubbing alcohol swab on site
    b. In spiral motion starting from the intended puncture site
    c. Up and down strokes of alcohol swab
    d. In spiral motion starting away from the intended puncture site

8. During venous puncture what is the correct angle of needle insertion into the vein?
    a. 15 - 30°
    b. 30 - 45°
    c. 45°
    d. Less than 15°

9. During venous puncture the needle should be inserted with:
    a. Bevel up
    b. Bevel down
    c. Bevel turned towards the left
    d. Bevel turned towards the right

10. What is the rationale for a particular sequence of blood specimen draw during collection of multiple samples using venous puncture?
    a. Minimize sample contamination
    b. Minimize hemoconcentration
    c. Minimize hemolysis
    d. Minimize platelet clumping
    e. Minimize clotting

11. Shaking the blood specimen tube forcefully to mix the contents may cause which of the following in the specimen?
    a. Hemo- concentration
    b. Hemolysis
    c. Hematoma
    d. Hemostasis

12. When transferring blood specimen from the syringe into an evacuated tube which one of the following explains correct technique?
    a. Remove the tube cap before transferring blood
    b. Hold the tube in hand and pierce the cap with needle
    c. Keep the tube on a rack, pierce cap with needle and let blood flow into the tube automatically
    d. Remove cap, remove needle from syringe and transfer blood into the tube
13. When should the tube be labeled?
    a. Before blood collection
    b. Before sending to the laboratory
    c. Immediately after collection
    d. Give sample to colleague for labeling

III. Phlebotomy Complications

1. Rolling of a vein is caused by?
    a. Puncturing through the vein
    b. Improper needle gauge used
    c. Excessive tourniquet pressure
    d. Improperly anchored vein
2. If puncture through the vein is suspected which of the following should be done?
    a. Observe for hematoma formation
    b. Remove the tube
    c. Pull the needle until it is in vein
    d. All of the above
3. If you observe formation of a hematoma during venipuncture?
    a. Ask patient to open the fist
    b. Release tourniquet pressure
    c. Apply pressure on site
    d. Cease venipuncture procedure immediately
4. Return of blood from an evacuated tube into a patient's vein during venous blood collection is called
    a. Pop off
    b. Vein collapse
    c. Reflux
    d. None of the above

IV. Capillary blood collection

1. What method of blood collection is preferred for infants?
    a. From the veins in hand
    b. Accessing indwelling lines
    c. Skin puncture
    d. Arterial puncture

2. Which of the following tests can't be performed on specimen collected by skin puncture ?
    a. Blood smear
    b. Blood culture
    c. PCR
    d. All of the above
3. Which of the following is the maximum length of lancet used for skin puncture on an infant's foot?
    a. 2.4 cm
    b. 3.0 mm
    c. 1.0 mm
    d. 2.0 mm
4. A potential complication of puncturing the bone during skin puncture, characterize by inflammation of the bone and bone marrow is called :
    a. Osteocarcinoma
    b. Osteopororsis
    c. Osteomyelitis
    d. Osteochondritis
5. Which of the following sites is the best site to select for skin puncture using patient's finger?
    a. Index finger
    b. Little finger
    c. Middle finger
    d. Palmer surface of any finger
6. Which of following tests are affected if povidine iodine is used for site cleansing before skin puncture?
    a. Potassium
    b. Phosphorus
    c. Uric Acid
    d. All of the above
7. Skin puncture performed on patient's finger should be made?
    a. At the crease between first and second phalanx
    b. On the tip of the finger
    c. Parallel to the fingerprints
    d. Perpendicular to the finger prints
8. How much times can the blood flow increase if the site is warmed before performing skin puncture?
    a. 4 times
    b. 2 times
    c. 7 times
    d. 10 times
9. Select the correct sequence of blood specimen draw during skin puncture.
    a. Red → Purple
    b. Purple → Red
    c. Any sequence

10. What is the rational for a particular sequence of blood specimen draw during skin puncture while collecting multiple samples?
    a. Minimize contamination
    b. Minimize hemolysis
    c. Minimize platelet clumping
    d. Minimize clotting
11. Which of the following could cause hemolysis of a specimen obtained by skin puncture?
    a. Collecting specimens in an incorrect order
    b. Alcohol left on skin before puncture
    c. Using iodine for cleaning the site
    d. Milking the site

V. Phlebotomy special techniques

1. Why is winged blood collection set recommended for venipuncture involving small and fragile veins?
    a. Bevel of the needle is longer
    b. Long tubing helps in better blood flow
    c. Reduces stress to vein hence prevents vein collapse
    d. Has sharper needle
2. Which one of the following statements is correct?
    a. Winged blood collection set can be used for evacuated collection
    b. Winged blood collection set can be used for syringe blood draw
    c. Winged set has flexible tubing
    d. All of the above
3. What is the angle of needle insertion while using winged blood collection set?
    a. 10 - 15°
    b. 15 - 30°
    c. 15 - 45°
    d. None of the above
4. Why is foot the last resort for blood drawing?
    a. Difficult to access
    b. Tougher skin
    c. Foot odor
    d. Risk of complications

VI. Safety and infection control

1. How can you be prepared to avoid a sharps injury?
    a. Organize equipment
    b. Keep sharps pointed away
    c. Keep safety box at arm's length
    d. All of the above

2. Under standard precautions, what personal protection equipment must the phlebotomist use for every blood collection procedure?
    a. Gloves
    b. Gown
    c. Gown, gloves, sharps and safety box
    d. Sharps and safety box
3. Which of the following are blood borne pathogens?
    a. HIV, HCV, HBV, HGV
    b. Herpes, Ebola, Dengue
    c. Brucella, Neisseria, Treponnema
    d. Mycobacterium, Mycoplasma, Toxoplasma
    e. All of the above
4. When should you change the safety box?
    a. Once it is completely full
    b. Every day
    c. If unable to insert more sharps
    d. Before it is full

VII. Specimen Transportation

1. What packaging system is used for specimen transport?
    a. Primary Tube System
    b. Single Pack System
    c. Double Pack System
    d. Triple Pack System
2. Serum / Plasma should be separated from cells before transporting to out-location -
    a. Within 2 hours of collection
    b. Within 24 hours of collection
    c. After receipt in the testing facility
    d. No need to separate
3. Whole Blood Samples for which of the following test (s) should be kept chilled?
    a. Lactic Acid
    b. Ammonia
    c. Parathyroid Hormone (PTH)
    d. All of the above
    e. Potassium
4. Person handling packed transport container should:
    a. Wear Gloves
    b. Not to carry in public transport
    c. Wear mask
    d. Keep the container upright

Guidelines for specimen collection & transport for laboratory testing during outbreak situation - Annexure K

Specimen collection & transport during outbreak investigation

Guidelines for specimen collection & transport for laboratory testing during outbreak situation

Integrated Disease Surveillance Project
National Centre for Disease Control

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Guidelines for specimen collection & transport for laboratory testing during outbreak situation - Annexure K

Introduction

Outbreaks of communicable diseases cause sufficient morbidity and mortality, consume scarce national resources, affect economic productivity and have the potential for International spread. They must be recognised and controlled rapidly in order to minimise their impact. The effective containment of an outbreak depends on:

- Early detection and reporting of suspect cases
- Rapid epidemiological investigation
- Rapid laboratory confirmation of the disgnosis
- The implementation of effective control measures

Rapid identification of the causative agent and the likely source or mode of transmission is essential. From this perspective, the initial investigation involves two important processes:

- Collection of information on suspect cases, and
- Collection of clinical specimens for laboratory diagnosis.

Successful lab confirmation of a disease depends on:

- Advance planning
- Collection of appropriate specimens
- Correct packagaing and rapid transport to an appropriate laboratory
- The ability of the lab o accurately perform the diagnostic test
- Proper biosafety and decontamination procedures to reduce the risk of further spread of the disease

The purpose of this document is to ensure the correct specimens are collected and transported in a safe and standardized manner during a field investigation of an outbreak.

Planning for specimen collection

Once a suspected outbreak has been detected and reported, an epidemiological investigation must be quickly organised. Laboratory investigations wherever applicable, are most useful to confirm the diagnosis but the epidemiological investigations should not be delayed until laboratory results are available. The materials and procedures required for efficient specimen collection and their transport to the laboratory for testing are outlined below.

1. Define the possible causes of the outbreak

An assessment of current clinical and epidemiological information is the starting point for considering the potential aetiology of the outbreak. The historical knowledge of regional, endemic and epidemic diseases, as well as their seasonality, further defines the possible causes. Since a variety of infectious agents can present with a similar clinical picture, the outbreak should be approached in a syndromic manner to obtain the differential diagnosis. One or more specimen types may be required to define the cause of the outbreak.

2. Decide which clinical specimens are required to confirm the cause of the outbreak

After defining the clinical syndrome and suspect pathogen(s), determine the clinical specimens for collection and appropriate laboratory diagnosis. This is best done in consultation with the laboratory(s) which will be performing the diagnostic testing. Review the sampling procedures and the necessary material.

3. Contact the laboratory for specimen testing

Contact the laboratory with appropriate capabilities. Key contact personnel from each referral lab must be nominated in advance who is responsible for:

- Coordinating the logistical aspects of sample handling and
- Transmitting information or queries between the field and the laboratory.

In consultation with the laboratory, organise all aspects of the handling of clinical specimens, from selection of sample type, collection materials, local or on-site processing, transport of specimens, and transmission of results should be organised in consultation with them. The laboratory support may be required for supply of special media, rapid kits etc.

4. Decide who will collect and transport the specimen

Decide whether a laboratory specialist/technician should join the team. Otherwise the team must receive training in collection, handling and transport of the required specimens as well as safety and decontamination procedures, the health workers joining the team during the course of investigation must also be offered this training.

5. Define the procedures necessary for specimen management

Consider the logistic requirements for sampling equipment and supplies, specimen handling and transport to the laboratory (timing route, transit temperature, requirements, shipping procedures and documentation) in advnace. In addition decide how the specimens would be transported and inform the receiving lab the approximate time at which they can expect the specimen.

6. Collecting the specimens

Specimen collection should commence as early as possible after a suspected outbreak has been notified. Specimens obtained in the acute phase of the disease, preferably prior to administration of antimicrobial drugs, are more likely to yield detectable concentrations of antibody, antigen or infective pathogen. Before beginning specimen collection, explain the procedure to the patient and relatives. When collecting the specimen avoid contamination and take a sufficient quantity of material (as guided by the laboratory tests). Follow the appropriate precautions for safety during collection and processing of specimens so as to protect the collector and lab personnel.

7. Safety

Healthcare workers may be unaware of potential etiologic agents residing in the specimen being transported to the laboratory. To protect the safety of the healthcare worker collecting the sample and the lab personnel, the following precautions must be followed when collecting specimens:

- During specimen collection wear personal protective equipment such as gloves, laboratory coat and where appropriate, a mask and / or goggles.
- Use leak-proof specimen containers and transport bags that have a separate outside compartment for the test requisition form.
- Never transport syringes with needles to the laboratory. Instead, transfer the contents to a sterile tube and place it in a sealable leak proof plastic bag.
- Make sure screw-cap lids are fastened evenly and securely. Ensure that no label material is caught in the threads of the lid.
- Do not transport leaking containers because test results will be compromised and it is a hazard to couriers and laboratory personnel.

- To protect the safety of others, take care not to contaminate the outside of the specimen container or the laboratory requisition form.
- Discard used needles directly into shraps container (puncture proof box) without recapping them
- Use 10% bleach to clean up spills after wiping the surface
- Contaminated non disposable equipment can be disinfectaed with 1% household bleach for 5 minutes and heavily soiled equipment can be soaked in 10% hosehole bleach before disposal

8. Labelling and identification of specimens

In an outbreak investigation the information contained in case investigation and laboratory request forms is collected along with the specimen. Each patient should be assigned a unique identification number by the collection team. It is the link between the laboratory results on the line listing form, the specimens and the patient, which guides further investigation and response to the outbreak. This unique identification number and the patient name should be present on all specimens, epidemiological data forms and the laboratory request and used as a common reference.

9. Labelling specimen container/slide

Labels should be used whenever possible. The label should be permanently affixed to the specimen container. It should contain the:

- Patient name
- Unique identification number
- Specimen type and
- Date and place of collection

10. Case investigation and laboratory forms

A case investigation form should be completed for each patient at the time of collection. The originals remain with the investigation team, and should be kept together for analysis and later reference. A laboratory form must also be completed for each specimen. The epidemiological and clinical data gathered in the investigation can later be easily tied to the laboratory results for analysis. The laboratory form includes patient information:

- Age (or date of birth), sex, complete address.
- Clinical information – date of onset of symptoms, clinical and immunisation history, risk factors, antimicrobial taken prior to specimen collection.
- Laboratory information – Acute or convalescent specimen, other specimens from same patient.
- The form records the date and time when specimen is collected, name of the person collecting the specimen.

The receiving lab should record the date and time when specimen was received, name and initials of the person receiving specimens, and arecord of specimen quality. The investigation team should receive a line listing form with the unique identification number and laboratory resuls for each specimen.

11. Storage of specimens

To preserve bacterial or viral viability in specimens for microbiological culture or inoculation, they should be placed in appropriate media and stored at recommended temperatures. These conditions must be preserved throughout transport to the laboratory and will vary according to transportation time. They will differ for specimens and pathogens, depending on their sensitivity to desiccation, temperature, nutrient and pH.

- Many specimens taken for viral isolation are viable for two days if maintained in type specific media at 4-8°C. For longer periods, freeze these specimens as directed by expert advice, as infectivity may be altered.
- Specimens for bacterial culture should be kept in appropriate transport media at the recommended temperature. This ensures bacterial viability while minimising overgrowth of other micro-organisms.
- With the exception of CSF, urine, and sputum, most specimens may be kept at ambient temperature if the specimen will be processed within 24 hours. For longer periods, storage at 4-8°C would be advisable with the exception of particularly cold-sensitive organisms, such as Shigella, Meningococcus, and Pneumococcus. Longer delays are not advisable as the yield of bacteria may fall significantly.
- Specimens for antigen or antibody detection may be stored at 4-8°C for 24-48 hours, or at –20°C for longer periods.
- Sera for antibody detection may be stored at 4-8°C for up to 10 days.
- Although not ideal, sera stored at room temperature may still be useful for antibody testing even after prolonged periods (weeks). Therefore, do not discard sera which have been collected simply because there are no refrigeration facilities available.

12. Packaging and labeling of specimens

Standardised packaging methods and materials ensure safety of personnel and specimen integrity, even if the package is damaged during transport. Laboratory request forms must accompany the labelled specimens. Specimens must be packaged, labelled and transported in compliance with specific national and international regulations for infectious materials.

Address labels on outer packages should display the sender and laboratory name with complete address and telephone numbers for both sender and receiver. Documentation should also contain specimen details (number, type, date of collection) appropriate biohazard labels, and the storage tempertaure requirements. Copies of letters, forms, permits and other identifying documents for the receiving laboratory should be placed together in aplastic bag and taped onto the outer transport packaging. A copy of these documents should also be given to the transport service.

13. Transport of specimens:

Before transport, the collection team should notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival.

General specimen collection guidelines

- Wash hands before and after the collection.
- Aseptic techniques must be employed during collection to prevent the sample from being contaminated during the process of collection.
- Avoid contamination from indigenous flora, whenever possible, to ensure a sample representative of the infectious process.
- Specimen should be collected before the administration of antimicrobial agents if possible (treatment for severe disease should not be delayed however).
- Collect the specimen at the appropriate stage of disease.
- Ensure that the correct specimen representative of the disease is collected
- Collect adequate volume, as insufficient material may yield false negative results.
- Collect or place the specimen aseptically in a sterile and appropriate screw capped container.
- Ensure that the outside of the specimen container is clean and uncontaminated.
- Close the container tightly so that its contents do not leak during transportation.
- Label the container appropriately and complete the requisition form.
- Arrange for immediate transportation of the specimen to the laboratory

Specimens To Be Collected

Syndrome	Possible agents	Specimen to be collected	Tests to be done
Acute Diarrheal Illness	Viral, Cholera, Shigella, Salmonella, E. coli, Amoeba, Giardia etc.	Faeces	•Stool microscopy •Stool culture and antimicrobial sensitivity •Genome detection
Acute Jaundice Syndrome	Hepatitis A – E, Leptospirosis	Blood. Urine (for Lepto)	•Serology •Blood for culture, virus isolation •Genome analysis
Acute Haemorrhagic Fever syndrome	Dengue, Hantavirus, Malaria	Blood CSF	•Blood smear examination •Virus isolation (Blood, CSF) •Antigen detection, antibody levels (CSF, Serum) •Genome detection
Acute Respiratory Syndrome	Influenza viruses, RSV, H. influenzae, Strep. Pharyngitis, Diphtheria, Pneumonic plague, etc.	Throat swab Sputum Serum Blood	•Culture and antimicorbial sensitivity (sputum, swab, blood) •Virus isolation •Antigen detection •Serology •Genome analysis
Acute Neurological Syndrome	Meningitis, Japanese Encephalitis, AFP, Rabies	Blood CSF Sera Faeces	•Gram stain for CSF •Antigen detection (CSF) •Culture/virus isolation (CSF, Blood) •Stool culture for viruses •Serology •Genome analysis
Acute Febrile Illness	Typhoid, Malaria, Hepatitis, Dengue, Leptospirosis, Anthrax, Brucellosis, Bubonic plague etc.	Blood Sera CSF Urine Bubo aspirate	•Gram stain •Culture (blood, urine, CSF) •Serology •Genome detection
Acute Dermatological Syndrome	Measles, Chicken Pox, Rubella, anthrax etc	Sera	•Serology •Culture •Genome detection

Feacal specimen collection

- Focal outbreaks of water borne and food borne diseases can occur throughout the year with a seasonal increase in the monsoon and post monsoon months. Establishing the aetiology of the diarrheal outbreak is an important part of outbreak investigation.
- Stool specimens are most useful for microbiological diagnosis if collected soon after onset of diarrhoea (for viruses < 48 hours and for bacteria < 4 days), and preferably before the initiation of antibiotic therapy. If required, two or three specimens may be collected on separate days, particularly useful when suspecting parasitic infections.
- Stool is the preferred specimen for culture of bacterial, viral and parasitic diarrhoeal pathogens.
- Rectal swabs showing faeces may be collected from infants (where collection of stool sample may not be possible). They are not recommended for the diagnosis of viruses.
- Collect diarrheal stool samples from at least 10 ill persons, if the number of ill persons is less than 10 then collect for all ill persons.

Materials for collection

- Clean, dry, leak-proof screw cap container and tape
- Appropriate bacterial transport media for transport of stool/ rectal swabs from infants
- Parasitology transport pack: 10% formalin in water
- Method of collecting a stool specimen
- Label the container
- Collect freshly passed stool, 5 ml liquid or 5 g solid (pea-size), in the container. (You may first ask the patient to pass the stool in a separate clean wide mouthed container and then transfer enough faeces with a spatula to the specimen container)
- Screw cap the container tightly and place in a sealed bag and send to laboratory immediately.
- In case of delay of more than two hours, the specimen may be transferred in refrigerated Cary Blair transport medium using two swabs. Cary Blair tubes with stool specimen must be transported to laboratory at 4 - 8°C and must be examined within 48hrs of collection.
- For viral specimens collect a larger volume of stool.

Method of collecting a rectal swab from infants

- Label the specimen tube/container containing the appropriate transport medium.
- Moisten a swab in sterile saline.
- Insert the swab tip just past the anal sphincter and rotate gently.
- Withdraw the swab and examine to ensure that the cotton tip is stained with faeces.
- Place the swab in the labelled sterile tube/container containing the appropriate transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Place in a sealed bag and send to laboratory immediately.

Handling and transport

- Stool specimens should be transported at 4-8°C. Bacterial yields may fall significantly if specimens are not processed within 1-2 days of collection. Shigella are particularly sensitive to elevated temperatures.
- Specimens to be examined for parasites should be mixed with 10% formalin- 3 parts stool to 1 part preservative and transported at ambient temperature in containers sealed in plastic bags.

How to transfer stool specimen into a tube containing Cary Blair transport medium

- Gloves to be worn at all times when handling the specimen.
- Take a sterile swab. Do not touch the cotton tip of the swab.
- Insert the cotton tip of the swab into the stool specimen.
- Make sure the cotton tip of the swab is completely coated with the specimen.
- Push the swab completely to the bottom of the tube of refrigerated Cary Blair transport medium.
- Break off the top portion of the stick so the cap can be tightly screwed onto the tube.
- After screwing cap tightly onto the Cary Blair tube, seal the tube with tape to prevent leakage.
- Adhere specimen label to the Cary Blair tube.
- Keep the tube at 4–8°C.
- Safely dispose of all contaminated materials.

Blood specimen collection

- Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable diseases.
- Venous blood can be used for isolation and identification of the pathogen in culture or separated into serum for the detection of genetic material, specific antibodies (by serology), antigens or toxins (e.g. by immunofluorescence).
- Clustering and sudden increase of acute cases of fever may be due to malaria, typhoid, dengue/chikungunya fever or other viral fevers. Fever with rash may be due to measles or chicken pox.
- In case of fevers suspected to be of viral aetiology, serum sample for detection of antibodies is to be collected. If clinical presentation is compatible with the case definition of typhoid fever, blood culture must also be collected.
- For the processing of most specimens for diagnosis of viral pathogens, serum is preferable to unseparated blood except where otherwise directed.
- When specific antibodies are being assayed, it is often helpful to collect paired sera, i.e. an acute sample at the onset of illness and a convalescent sample one to four weeks later.
- Blood can also be collected by fingerprick for the preparation of slides for microscopy or for absorption onto special filter paper discs for analysis.
- Whenever possible, blood specimens for culture should be taken before antibiotics are administered to the patient.
- If suspecting Measles: Collect blood between the 3rd and 28th day after onset of rash from at least 5 to 10 suspected cases, for confirmation of an outbreak.
- If suspecting Viral hemorrhagic fevers: Collect from the first suspected VHF case. If more than one suspected case present, collect until diagnosis is confirmed.

Venous blood samples

Materials for collection

- Skin disinfection: 70% alcohol (isopropyl alcohol, ethanol) or 10% povidone iodine, swabs, gauze pads, band aid.
- Disposable latex or vinyl gloves
- Tourniquet, Vacutainer or disposable syringes and needles.
- Vacutainer or sterile screw cap tubes (or cryotubes if indicated),
- Blood culture bottles (50ml for adults, 25ml for children) with appropriate media.
- Labels and indelible marker pen.

Method of collection

- Gloves to be worn at all times when handling the specimen.
- Disinfect the tops of blood culture bottles.
- Place a tourniquet above the venepuncture site.
- Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venipuncture site outwards. Let the disinfectant evaporate. Do not palpate the vein again.
- Perform venipuncture.
- If withdrawing with conventional disposable syringes, withdraw 2-3 ml venous blood and transfer to small blood collection tubes/vials for serological tests.
- For blood culture withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants. Using aseptic technique, transfer the specimen to relevant culture bottles or blood collection tubes/vials for serum.
- If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each culture bottle or vacutainer.
- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply bandaid.
- Label the tube, including the unique patient identification number, using permanent marker pen.
- Do not recap used sharps. Destroy the needle using the needle destroyer and discard the remaining syringe directly into the appropriate container for infectious plastic waste.
- Complete the case referral forms.

Handling and transport

- Blood specimen bottles and tubes should be transported upright and secured in a screw cap container or in a rack in a transport box. They should have enough absorbent paper around them to soak up all the liquid in case of spill.
- Blood cultures-If the specimen will reach the laboratory within 24 hour, most pathogens can be recovered from blood cultures transported at ambient temperature. Keep at 4-8°C for longer transit periods, unless one suspects a cold-sensitive bacterial pathogen (eg meningococcus).

How to separate serum from whole blood

- Gloves should be worn at all times when handling the specimen.
- Keep the whole blood at room temperature until there is complete retraction of the clot from the serum.
- If a nearby health facility has a centrifuge, spin the whole blood at 1000 x g for 10 minutes to separate the serum. If centrifuge not available, serum can still be separated carefully.
- Remove the serum using a sterile pipette. Avoid extracting red cells.
- Transfer the serum aseptically to a sterile, screw-capped prelabelled tube.
- Secure cap tightly.
- Safely dispose of all contaminated materials and the remaining clot.
- Keep the tube of serum at 4–8°C.
- If facilities for separation of serum are not available, then the clotted sample should be stored at 4 to 8 °C (NOT FROZEN). Protect such sample from excessive vibration while transporting.

Cerebrospinal fluid (CSF) specimen collection

- Meningitis outbreaks may occur due to bacterial or viral organisms. A lumber puncture for demonstration and/or isolation of organism form CSF is essential. Commercially available latex agglutination kits may provide diagnosis of some agents of bacterial meningitis.
- The specimen must be taken by a physician or a person experienced in the procedure. CSF is used in the diagnosis of viral, bacterial, parasitic, and fungal meningitis/encephalitis.

Materials for collection

Lumbar puncture tray which includes:
Sterile materials: gloves, cotton wool, towels or drapes.
Local anaesthetic, sterile needle & syringe.
Skin disinfectant: 10% povidone iodine or 70% alcohol.
Two sterile lumbar puncture needles, small bore with stylet.
Small sterile screw-cap tubes and tube rack.

Method of collection

As **only experienced** personnel should be involved in the collection of CSF samples, the method is not described in this document. CSF is collected directly into the separate screw-cap tubes. If the samples will not be promptly transported, separate tubes should be collected for bacterial and viral processing.

Handling and transport

- In general, specimens should be delivered to the laboratory and processed as soon as possible.
- CSF specimens for bacteriology are transported at ambient temperature, generally without transport media. They must never be refrigerated as these pathogens do not survive well at low temperatures.
- CSF specimens for virology do not need transport medium. They may be transported at 4-8°C for up to 48 hours, or at -70°C for longer periods

Respiratory tract specimen collection

- Specimens are collected from the upper or lower respiratory tract, depending on the site of infection. Upper respiratory tract pathogens (viral and bacterial) are found in throat and nasopharyngeal specimens. Lower respiratory tract pathogens are found in sputum specimens. For organisms such as Legionella, culture is difficult, and diagnosis is best based on the detection of antigen excreted in the urine.
- When acute epiglottitis is suspected, no attempt should be made to take throat or pharyngeal specimens since these procedures may precipitate respiratory obstruction. Epiglottitis is generally confirmed by lateral neck X-Ray, but the etiologic agent may be isolated on blood culture.
- For severe hospitalized pneumonia cases, blood culture may be an appropriate test (see above for collection of blood for culture)

Materials for collection

- Transport media – bacterial and viral
- Dacron and cotton swabs
- Tongue depressor
- Flexible wire calcium alginate tipped swab (for nasopharyngeal swab)
- Nasal speculum (for nasopharyngeal swab – not essential)
- Suction apparatus or 20-50 ml syringe
- Sterile screw-cap tubes, and wide-mouthed clean sterile jars (minimum volume 25ml)
- Plastic catheter,
- Sterile normal saline

1. Upper respiratory tract specimens

Method of collecting a throat swab

- Label the specimen containers.
- Hold the tongue down with the depressor.
- Use a strong light source to locate areas of inflammation and exudate in the posterior pharynx and the tonsillar region of the throat behind the uvula.
- Rub the area back and forth with a Dacron or calcium alginate swab. Withdraw the swab without touching cheeks, teeth or gums and insert into a screw cap tube containing transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Complete the case referral form.

Method of collecting nasopharyngeal

- Label the specimen tube.
- Seat the patient comfortably, tilt the head back.
- Insert a flexible calcium alginate/Dacron swab through the nostril to posterior nasopharynx (same distance as from nostrils to external opening of ear) parallel to the floor of nose without pointing upwards.

- Rotate the swab on the nasopharyngeal membrane a few times to obtain infected cells.
- Repeat procedure using other nostril. Collection of specimens from both nostrils increases the amount of material for analysis and the ability to isolate the virus.
- Place the swab(s) in the tube of viral transport medium.

Method of collecting nasal swabs

- Seat the patient comfortably, tilt the head back
- Insert cotton bud end of dry sterile swab into right nostril (approx. 2 cm inside) and rub firmly against the turbinate (to ensure swab contains cells as well as mucus)
- Insert swab into a screw cap tube containing transport medium. Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Repeat procedure for left nostril using new sterile swab and insert into same tube of transport medium
- Complete the case referral/lab request form.

Method of collecting Nasopharyngeal wash/aspirate

- Label a sterile vial
- Ask the patient to sit with the head tilted back.
- Flush plastic catheter with 2-3 ml VTM/sterile normal saline.
- Instil 1-1.5 ml of VTM/sterile normal saline into one nostril.
- Insert tubing into this nostril parallel to the palate and aspirate secretions.
- Repeat the procedure with the other nostril.
- Collect 1-2 ml in the labelled sterile vial and transport at 2-8 ^{0}C.

2. Lower respiratory tract specimens

Method of collecting sputum

- Label the specimen container.
- Instruct patient to take a deep breath and cough up sputum directly into a wide-mouth sterile container. Avoid saliva or postnasal discharge and avoid soiling the outer walls of the container.
- Good sputum specimen should contain the thick purulent material, and be at least 1 ml in amount.
- Complete the case referral form/laboratory request form.

Handling and transport

- All respiratory specimens except sputum are transported in appropriate bacterial/viral media.
- Transport as quickly as possible to the laboratory to reduce overgrowth by commensal oral flora.
- For transit periods up to 24 hours, transport bacterial specimens at ambient temperature and viruses at 4-8°C in appropriate media.