

Ethiopian Public Health Institution

National HIV Reference Laboratory



Specimen Collection, Handling, Transportation and Storage Manual

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REVISION AND AMENDMENT

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Abbreviations

ACTH	Adreno Cortico Tropic Hormone
AFP	Alpha-Fetoprotein
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Asparate Aminotransferase
B-HCG	Beta Human Chorionic Gonadotropin
CEA	Carcino Embryonic Antigen
CK	Creatine kinas
CSF	Cerebrospinal fluid
DHEA-S	Dehydroepiandrosterone sulphate
DBS	Dried Blood Spots
EPHI	Ethiopian Public Health Institute
ELISA	Enzyme linked immunosorbant assay
FBS	Fast blood sugar
FMHACA	Food medicine and health care administration and control authority of Ethiopia
F-PSA	Free Prostate-Specific Antigen
FSH	Follicle-Stimulating Hormone
FT3	Free Triiodothyronine
FT4	FreeTriiodothyronine
HCL	Hydrochloric acid
HDL	High density lipoprotein



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ID	Identification card
IV	Intravenous
LDL	Low density lipoprotein
LIS	Laboratory information system
MOH	Ministry of health
NA	Not applicable
NHIVRL	National HIV Reference Laboratory
PCR	Polymerase chain reaction
PTH	Parathyroid Hormone
RT	Room temperature
SOP	Standard operating procedures



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Introduction

Proper specimen collection and handling is an integral part of obtaining a valid and timely laboratory test result. Specimens must be collected in an appropriate specimen container to maintain the integrity of the specimen. Test information sheets specify the type of container or collection kit that should be used to collect the sample. During collection, specimens must be labeled with the patient's full name (or unique code number in the case of anonymous testing) and If possible barcode generated labeling which contain patient Name, Patient ID, date and time of sample collection is mandatory.

Specific handling or storage information is included in the test-specific kit instruction sheets and test information sheets. All specimens must be packaged carefully to avoid breakage or leakage of the specimen.

The packaging and transportation of all diagnostic specimens or biological products must comply with the transportation of dangerous goods regulations. All packages sent to the NHIVRL must be constructed, filled, closed and secured so that under normal conditions of transport, including handling, there will be no accidental release of the substance that could endanger public or employee safety.

The NHIVRL ensures that quality specimens are used for testing in all of its laboratories. All specimens should be collected by a qualified and trained professionals in accordance with test-specific instruction contained in the manual. Each laboratory develops and implements test specific criteria to ascertain compliance with the policy. The NHIVRL, through its laboratory response unit, ensures that copies of this manual are at all times accessible to and are understood by all professionals engaged in specimen collection for tests done at the institute. The NHIVRL is responsible to oversee the overall implementation of this manual.



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Common errors during specimen collection

- Failure to label a specimen correctly and provide all pertinent information required on the test request form.
- Insufficient volume of specimen to run test (critical samples might be considered exceptionally eg: CSF)
- Failure to use the correct container
- Test tube with inappropriate specimen preservative.
- Incomplete patient instructions prior to collection.
- Failure to tighten specimen container lids, resulting in leakage and/or contamination of specimens.
- Inappropriate way of sample transportation and package.

In addition all specimens should be collected and handled with universal precautions, as if it is hazardous and infectious. The quality of the specimen obtained in the pre-analytical phase of testing is crucial to the output of accurate and reliable results. “Garbage in = Garbage out.”

In order to have appropriate sample collection, the following important processes should be followed:

1. Proper patient identification procedures.
2. Proper equipment and materials selection
3. Proper labeling procedures and completion of laboratory requisitions.
4. Order of draw for multiple tube phlebotomy.
5. Safety and infection control procedures

In addition, identify the additive, additive function, volume, and specimen considerations to be followed for each of the various color coded tubes.

All specimens delivered to the laboratory must meet defined acceptance criteria for identification/labeling, collection, volume, preservation, and container type in order to be processed. If any criteria are not met, the NHIVRL should communicate with responsible person and immediate corrective action should be taken.



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Specimen rejection criteria:-

- a) Specimen collected in the wrong tube, container and preservative.
- b) Specimen inappropriately handled with respect to temperature, timing or storage requirements.
- c) Insufficient volume of specimen (critical samples might be considered exceptionally e.g. CSF or body fluid)
- d) Lipemic or grossly hemolyzed specimens may be rejected depending on test requested.
- e) Specimens with IV fluid or other peripheral line contamination
- f) If the specimen collection device are expired.
- g) Specimens submitted in syringes with needles are considered unacceptable.
- h) Specimens submitted in cracked or leaking containers with external contamination of blood/body fluids.

Labeling of Specimen

Specimen should be labeled with appropriate codes and should contain all essential information regarding to the patient. If any specimen is unlabeled, mislabeled, or improperly or incompletely labeled, it may be rejected and also the NHIVRL communicate with laboratory response unit and any concerned health facility to properly labeled and resend specimen. The reason for the rejection must be documented on the specimen rejection log.

Laboratory Requisition

All laboratory specimens must be accompanied by an adequate requisition for the test. Paper or electronic requisitions must include the following:



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1. Adequate patient identification information,(patient Name)
2. Sample ID
3. Patient sex,
4. Date of birth or age,
5. Name of physician or legally authorized person ordering the test,
6. Tests requested,
7. Time and date of specimen collection
8. Initials of person collecting the specimen
9. Source of specimen, and
10. Clinical information when appropriate.

Specimen Collection and Receipt Flow Chart:-

The following flow chart depicts the sequence of activities conducted at Laboratory reception unit

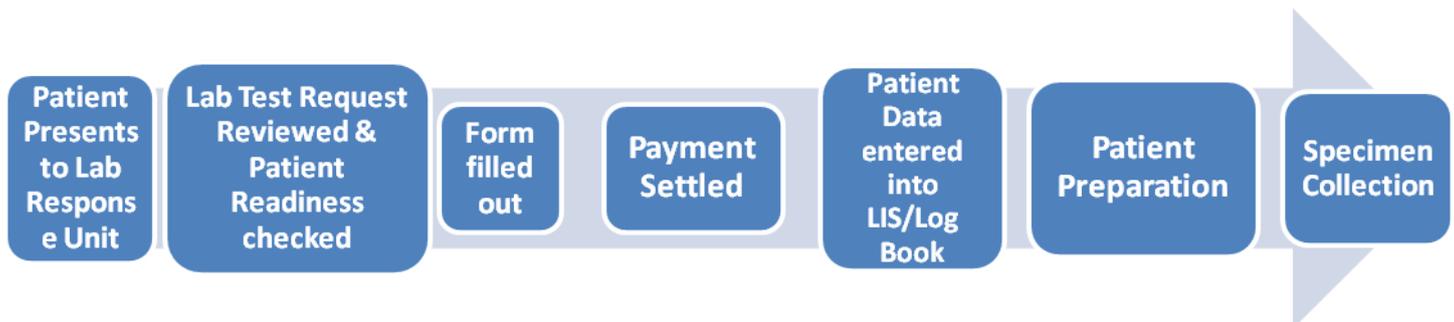


Fig 1: Path of work flow for pre-specimen collection procedure at the Laboratory reception unit.

Patient Preparation

If the patient preparation procedure fails to lead to specimen collection or in case of unsuccessful specimen collection, appropriate arrangement should be made for the next step towards effective sample collection.

As shown on the figure 1 above, The Path of work flow for pre-specimen collection procedure at referral laboratory.



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1. Patient presents to laboratory reception unit,
2. Lab test request reviewed on the test availability, appropriateness of collection time (e.g. Cortisol and other hormonal tests), patient readiness for collection (fasting, morning urine, acceptance of appointment),
3. Request paper properly filled out based on the identified tests to be performed
4. Payment settlement:
 - Cash
 - Credit service: to the staffs who can bring confirmation letter (two copies) from an organization that have prior agreement with EPHI
 - Free service registration for clients who have free service letter/ID
5. Patient data entered into LIS/Log Book
6. Patient preparation – follow laboratory sample collection manual
7. Specimen collection based on the procedure in the specific SOPs



Fig 2: Path of work flow for referral specimen receipt at the Laboratory reception unit.

1.1 Patient preparation for clinical chemistry tests

1. Adrenocorticotrophic hormone, ACTH

- The patient should consume a low carbohydrate diet for 48 hours before specimen collection
- Fasting for 10-12 hours and limited physical activity before specimen collection is required.

2. Cortisol

- Fasting and no exercise for 10 to 12 hour are required prior to specimen collection.
- As the standard the blood must be collected between 8:00 to 8:30 AM only, but specimen may be collected at any other time if requested by the physician.



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3. Parathyroid Hormone, PTH

- Fasting for 8 to 10 hours is required before specimen collection

4. Insulin

- Fasting for 8 hours is required prior to specimen collection. Water is permitted.

5. Fasting Blood Sugar [FBS]

- Fasting of at least 8 hours is required prior to specimen collection but water is permitted.
- Random Blood Sugar do not need of fasting.

6. Total Cholesterol

- Fasting for 12 hours is required prior to specimen collection but water is permitted.
- No alcohol is allowed for 24 hours prior to specimen collection.

7. High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Triglycerides and Transferrin

- Fasting for 12 hours is required prior to specimen collection but water is permitted.

8. Creatine Kinase (CK), MB and Creatine Kinase Total, CK

- No intramuscular injections for 1 hour and intensive exercise prior to specimen collection

9. Urea

- A diet with high red meat should be avoided for 12 hours prior to specimen collection.

10. Uric acid

- Fasting is usually required for 8 hours prior to specimen collection
- Avoid intake of meat and fish 12 hours prior to specimen collection

11. Vitamin B12 or Cyanocobalamin

- 12 hours fasting preferred; must draw before Schilling's test, transfusions or B12 therapy is started.

12. Folic Acid (Folate)

- Fasting is required for 8 hours prior to specimen collection. Water intake is allowed. No alcohol is allowed prior to specimen collection

13. Prolactin (PRL), Follicle-Stimulating Hormone (FSH),Luteinizing Hormone (LH) Progesterone (PROG), Estradiol (E2) and Testosterone (Testo)

- No fasting is required prior to specimen collection.
- The patient should rest 30 minutes prior to specimen collection.
- The sample should be drawn in the morning

Note: All other blood chemistry tests do not require special patient preparation for testing.



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1.2 Patient preparation for Immunology and Hematology test

No special patient preparation required for Floctometry (CD4 Count) and Hematology (CBC) tests. However to avoid diurnal variation in CD4 count, the CD4 should be collected at same time always (preferably in the morning).

1.3 Patient preparation for Molecular tests

No special patient preparation required for HIV Viral load test and DNA PCR test (Early Infant Diagnosis (EID))

Specimen collection materials

- Swabs
- Gloves
- Tourniquet
- Vacutainer holder
- Syringe with needle
- Vacutainer needles
- Pediatric blood collection tube (K₂ or K₃)
- Pediatric blood collection tubes
- Whatman 903 Dried Blood Spots (DBS) card
- A tube rack which fits the Vacutainer tubes
- Sharps disposing Container
- Vacutainer Tubes, plain/red-top/SST
- Tube rack which fits the Vacutainer tubes
- Gauze Sponges
- First Aid Plaster
- Sharps disposing Container
- Waste disposing Container
- EDTA Tube (K₂ or K₃)
- Gauze Sponges
- Sealable plastic bags
- butter fly needle
- Waste disposing container
- Red top or Serum-separating tubes



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Specimen Collection Procedure:-

1.4 Specimen collection for whole blood

Step	Action
1	Identify the patient by asking the name of the patient and ensure the name matches on the requisition form
2	Assemble necessary specimen tubes and supplies
3	Complete the specimen container with the Patient ID, name of health facility, date and time of collection
4	Seat the patient in a comfortable chair with the arm on a sturdy support
5	Explain the procedure to the patient and reassure the patient of their safety
6	Tie the tourniquet 2 inches above the anticipated puncture site and palpate the vein. The tourniquet should be positioned 7.5Cm to 10Cm above the puncture site. Do not leave tourniquet on for extended period of time (> 1 minute)
7	Clean the area with alcohol pad in a circular motion starting from the center and working outward. Wipe the area with dry gauze square. Do not touch after cleaning.
8	uncap and insert the needle bevel up into the vein and observe the blood is coming
9	Gently slide the tube into the specimen holder and withdraw peripheral blood one third of standard appropriate test tube.
10	Loosen the tourniquet, place sterile gauze over the needle at the puncture site and remove the needle. Using gauze apply gently pressure to the puncture site until bleeding stops. Tape the gauze firmly to the arm.
11	Gently mix the blood properly by inverting the tube five to ten times, immediately after collection to avoid formation of small clots (This is not practical for whole blood collected with serum separate test tube)
12	Discard the used materials in appropriate safe container and tell the client to do so
13	Double check the tube labels for accuracy with the sample request form before sending the sample to the lab
14	Inform the patient on the turnaround time of each test and appointment date



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15	Thank the patient for his/her collaboration
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1.5 Sample Collection procedure for dried blood spot (DBS)

Step	Action
1	Wash hands and put on a new pair of powder-free disposable gloves. NOTE: A new pair of gloves must be used for each patient.
2	Clearly label the Whatman 903 Card with Patient ID (MRN) and/or name of infant, date of collection
3	Select the lateral side of the fingertip or great toe or heel based on age and weight of infant NOTE: Warming the skin-puncture site can increase blood flow.
4	Disinfect selected site and prick using lancet/needle NOTE: Clean the selected area with 70% alcohol and allow the area to air dry for 30 seconds. Alcohol residue remaining on the skin may dilute the specimen and adversely affect test results.
5	Wipe away first drop of blood with sterile, dry gauze as this contains tissue fluids that can affect the results.
6	Apply gentle pressure and allow a large drop of blood and collect onto the middle of the circle on the DBS card and continue this process until 4- 5 circles are filled. NOTE: Don't touch the Whatman 903 DBS Card at any stage of collection.
7	Clean the area and leave with no bandage after the required numbers of spots have been filled.
8	Place the completed DBS card horizontally without touching the spots on a drying rack for a minimum of 3 hours or overnight to allow the blood spot to completely dry NOTE: Keep the drying cards away from direct sunlight and do not heat, stack, or allow the spots to touch other surfaces during the drying process. Touching or smearing the blood spots must be avoided. Care must be taken to prevent insects or mice from contaminating and deteriorate the DBS cards. Dry completely before packaging.
9	Wrap each DBS card with a folded sheet of glassine paper and place the glassine paper-wrapped cards into a low gas-permeable, zip closure plastic bag
10	Store 10-15 individually wrapped cards in a humidity proof bag. Each bag should have 10 desiccant bags and 1 humidity indicator card.



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11	Keep packaged DBS at room temperature until transported to referral laboratory. There is no need of refrigeration
12	Clearly label the outside of each bag with content information such as the range of lab ID numbers contained in the bag
13	Place 10 bags in a cardboard box (13X13cm). On each box, write the box number, the range of lab ID numbers, and number of bags.
14	Transfer this information to the lab data log. Place the boxes in a -20 ⁰ C freezer that is dedicated for sample storage

1.6 Urine Sample collection procedure for clinical chemistry and electrolyte tests

Steps	Action
1	Provide a sterile container to a patient
2	Instruct the patient to void several milliliters and collect midstream urine without stopping
3	For catheterized specimens, a urine sample is taken by inserting a thin rubber tube or catheter through the urethra into the bladder. The urine is collected in a sterile container at the other end of the tube
4	Label the container clearly with the patient name or patient ID number; date of collection and time the patient passed the urine

Specimen packaging and transport:-

1.7 Specimen packaging

The packaging of specimens must consist of three components to comply with standard regulations:

- (a) A primary receptacle – e.g. the container or blood tube a specimen is collected into;
- (b) Secondary packaging – e.g. the purpose designed plastic specimen bag
- (c) An outer packaging – e.g. the Versapak bag used to transport specimens to the laboratory.

There should be absorbent material present in the outer packaging to absorb potential spills and leakages.

Specimen containers must be tightly sealed to render them leak proof. Specimens and request forms must be placed in purpose designed plastic specimen bags. The specimens must be placed in the specimen chamber which must be sealed, the request form placed in the side pocket.



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Specimens and request forms must not be put in the same compartment in case of leaking sample containers.

- (a) Clinical chemistry, HIV viral load, CD4 and Haematology samples can be sent together, in the same specimen bag. Dried blood spot for DNA PCR should be kept separately.
- (b) Although all samples should be treated as *HIGH-RISK*, (standard precautions), known high risk specimens should be labelled as ‘High Risk’
- (c) Specimens should be transported to the laboratory using the supplied standard compliant carriers, e.g. triple pack transport bags. These are secure; contain an inner, replaceable lining for maximum sample & staff protection and safety and absorbent material to soak up any potential leakages. Various sizes are available for individual or multiple specimens.
- (d) When using these carriers there is no need for the person transporting the container to wear gloves or face protection.

It is the prime responsibility of the user/sender to collect and package specimens according to the relevant guidelines. The NHIVRL reserves the right to refuse acceptance of patients’ specimens, not packaged in accordance with current guidelines which pose a hazard to its staff, couriers or other health care workers.

1.8 Specimen transportation

Responsibility for Transportation:-

Ethiopian postal service is responsible for providing transportation services in the Primary and Secondary care environments. The EPHI regional laboratory capacity building directorate have good links with Ethiopian postal service which enable cooperation between the two services for timeliness and safety considerations.

The EPHI regional laboratory capacity building directorate are responsible for training of porters and drivers in the nature of pathology specimens and health & safety requirements, including dealing with spillages.

After specimen received from referring laboratory to EPHI central laboratory response unit and collected on site should be distributed by assigned responsible person to each laboratory according to the test to be performed.



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Ensuring the Safety of the General Public:-

For any breakage or spillage of one or more specimens which are in an area that the general public have access to, e.g. ward and clinic areas or public corridors, the following guidance should be followed.

The person who has dropped the specimen or noticed that the specimen was leaking should contact the responsible person, he/she should try to ensure that the area is kept clear from all staffs, members of the general public, relatives and patients, by enlisting help if possible and available.

A representative from the specimen sending department should go to the site where the leakage/breakage occurred and should notice if one of their blood tubes is involved.

The responsible body should assess the situation and the relevant risks if any associated with the specimen.

Monitoring of Transportation of Specimens:-

Regular audits are carried out of the transport arrangements of specimens to the laboratory from referral sites according to documented laboratory procedure.

Time & Temperature

These audits are undertaken to ensure that specimens are

- I. Transported within a time frame appropriate to the nature of the requested tests (i.e. making sure there is no undue delay in getting samples to the laboratory. This is accepted to be between 4 and 5 hours) and
- II. Transported within a temperature range between 10 and 28°C to ensure sample integrity.

Specimen Container Preservatives:-

Continuous, real time monitoring of specimens occurs when they are received in the laboratory. This monitoring check for the correct specimen container and volume of specimen for the requested tests as well as completeness of patient ID.

Identification of Compromised Specimen Integrity or Unsafe Packaging

In all instances where, upon receipt of a sample whose integrity was compromised or it has come to the attention of laboratory staff that specimen packing or transportation practices could have or



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did jeopardise the safety of the courier or the general public, the laboratory will investigate the issue and contact the sender of the specimen to inform them about measures to be taken to minimise or where possible, eliminate recurrence.

Depending upon the seriousness of the incident, laboratory staff may raise an occurrence report (e.g. spillage/leakage of specimen in a public place) and communication with the sender may be either by telephone call or by way of a comment.

Reporting of Incidents Relating to Specimen Packing & Transportation

Any adverse incident which occurs in the course of dispatching and transporting clinical specimens to the laboratory must be recorded using the NHIVRL incident reporting system.

Examples of types of incidents include:-

- Leaking specimens and associated contamination
- Serious delay in transportation of specimens
- Breakdown of vehicles transporting specimens
- Incorrect storage of specimens prior to transportation

Summary

Below is the summary of; type of container required, transportation requirement, and storage condition and specimen stability.

Type of test	Container	Volume	Transport and packaging	Storage	Stability
Whole blood					
EID	DBS	4-5 spots	At ambient to and shipped within a week to two weeks from collection site	Room To and - 20 ° C	Stable for 3 months at room temperature and for longer period at -20 °c and colder
Hematology	EDTA tube (K ₂ or K ₃)	3-5 ml of whole blood	At room temperature	Room temperature or /2-8°c	8 hours
CD4	EDTA tube (K ₂ or K ₃)	>1/3 of the standard K ₂ or K ₃ EDTA tube	At room temperature	Room temperature	for 48 hours





RBC foliate	Na-heparin or K3-EDTA	3-5 ml of whole blood	The sample transported using triple package system at room temperature and the sample is must be covered with aluminum foil.	At Room temperature	2 hours
				At 2-8°c	24 hours
				At -20 °C	1 month (only EDTA-blood)
Tina-quant Hemoglobin A1c	Li-heparin, K2-EDTA, K3-EDTA, Fluoride/Na2-EDTA, Na-Heparin ,Fluoride/potassium oxalate.	3-5 ml of whole blood	The sample transported using triple package system at room temperature	At room temperature	3 days
				at 2-8°C	7 days
				at -20°C	6 months
ACTH	K2- and K3-EDTA:	3-5 ml of whole blood	Only use pre-cooled sampling vials. After drawing the blood, put the vials immediately on ice. Use a cooled centrifuge to separate the plasma. Measure samples immediately or freeze them at -20 °C.	At room temperature	2 hours
				-20 °C	4 weeks (Freeze only once)
Vitamin B12 & Folate	SST or Na-heparin, Li-heparin, K2-EDTA and K3-EDTA	3-5 ml of whole blood	The sample transported using triple package system at room temperature and the sample is must be covered with almunium foile.	At room temperature	2 hours
				2-8 °C	48 hours
				(-15)- (-25) °C	56 days Freeze once only.
Protein Urine	Random Urine or 24 hour Urine Use no preservatives. Refrigerate specimen during collection	For Random Urine(up to 5 ml Urine)	The sample transported using triple package system at room temperature	At room temperature	1 day
				At 2-8 °C	7 days
				At (-15)-(-25) °C	1 month
Protein CSF	Use no preservatives.	Up to 1 ml	At room temperature	At room temperature	1 day





	Refrigerate specimen during collection			At 2-8 °C	6 days
				At (-15)-(-25) °C	> 1 year
Urine Glucose	Dark bottle/ 5 mL of glacial acetic acid to the container.	For Random Urine up to 5 ml	At room temperature	Store immediately at 2-8 °C and (-15)-(-25) °C	analyzed for glucose immediately
CSF Glucose	Sterile Tube	Up to 1 ml	At 2-8 °C	at 4 °C or -20 °C	analyzed for glucose immediately
All Clinical chemistry tests	Serum separating tube	3-5 ml of Whole Blood	Transport at room temperature	Only room temperature	at least for 1 hour at room temperature
Viral load	EDTA tube	4-5 ml	Triple package at room temperature	At room temp	For not more than 6 hours.
	Plasma separating tube			2-8°C	For 24 hours
Plasma					
Viral load	Nunk tube	1.8ml to 2ml	Triple package at room temperature	2°C to 8°C	for 5 days
				-20°C	For 1 month
				- 80°C	for longer period

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- Standard Operating Procedure for Specimen Collection, Handling and Transport for Hematology and CD4 testing; HS-001: EPHI.
- Collection, storage and shipment of Dried Blood Spots (DBS) for Early Infant Diagnosis (EID) DNA - PCR Testing Document Number: HIV-TP#07
- Amplicor HIV-1 DNA Test, Version 1.5 kit insert
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Annex



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Annex-1 General Laboratory Safety Precautions

- All staffs involving in handling of laboratory specimens should receive safety training and take appropriate vaccination. Wear protective clothing and gloves when processing blood and body fluid specimens or when performing some procedures like Vein puncture and other vascular access procedures.
- Employ appropriate barrier precautions to prevent skin and mucous membrane exposure
- Take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments.
- Do not recap, bend or break needles by hand or remove needles from disposable syringes.
- Discard all sharp instruments in puncture-resistant containers located close to work area.-
- Secure lids immediately, to avoid spillage and contamination during transport.
- Place all liquid specimens in containers that prevent leakage during transport
- Limit use of needles and syringes to situations in which there is no alternative
- If hands or other skin surfaces become contaminated with blood or other body fluids, wash them immediately and thoroughly with soap and water.
- Remove gloves and wash hands with soap and water upon completion of specimen processing after contact with each patient.
- Employ a biological safety cabinet for procedures that have high potential for generating droplets.
- Use mechanical pipette devices to manipulate all liquids in the laboratory.
- Decontaminate laboratory work surfaces at least daily with a freshly prepared chemical germicide such as a 1:10 dilution of household bleach
- Disinfect refrigerators by cleaning thoroughly and then by wiping with 1:10 dilution of household bleach.
- Disinfect centrifuge components by swabbing head, bowl and carriers with 70% ethanol.
- Autoclave or soak specimen racks in 1:10 dilution of household bleach for 5 minutes and then rinse thoroughly with water.



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- Discard as hazardous waste any disposable components of instrument systems that come in contact with patient specimens and clean non-disposable components with 70% ethanol.
- Allow disinfectant to remain in contact with surfaces for at least 5 minutes at ambient temperature for optimal effectiveness against dried blood or serum.
- If equipment needs maintenance, clean and decontaminate it in the laboratory before transporting it to the manufacturer for repair.
- Incinerate or autoclave all waste before disposal in a sanitary landfill. Solutions containing bleach may corrode the autoclave; therefore, these solutions may be poured down a drain connected to a sanitary sewer.
- After decontaminating, carefully pour down a drain connected to a sanitary sewer bulk blood, suctioned fluids, excretions, and secretions.
- Decontaminate spills of blood and body fluids by
 - ✓ wearing disposable gloves,
 - ✓ Covering visible blood or body fluids with paper towels and soak it with a 1:10 dilution of household bleach. Allow to stand for at least 5 minutes.
 - ✓ Discarding contaminated towels in infectious waste containers.
 - ✓ Wiping down the area with clean towels soaked in a 1:10 dilution of household bleach.

Any specimen should be submitted to the laboratory with appropriate handling procedures. Any specimen submitted in a manner which could create a health or safety hazard to laboratory personnel is considered unacceptable.



