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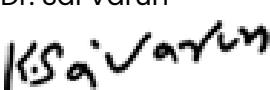
where Trust matters

**SPECIMEN COLLECTION, TRANSPORTATION
& HANDLING MANUAL**

2025

**SPECIMEN COLLECTION,
TRANSPORTATION & HANDLING MANUAL**

APPROVAL AUTHORITY

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RECORD OF AMENDMENTS

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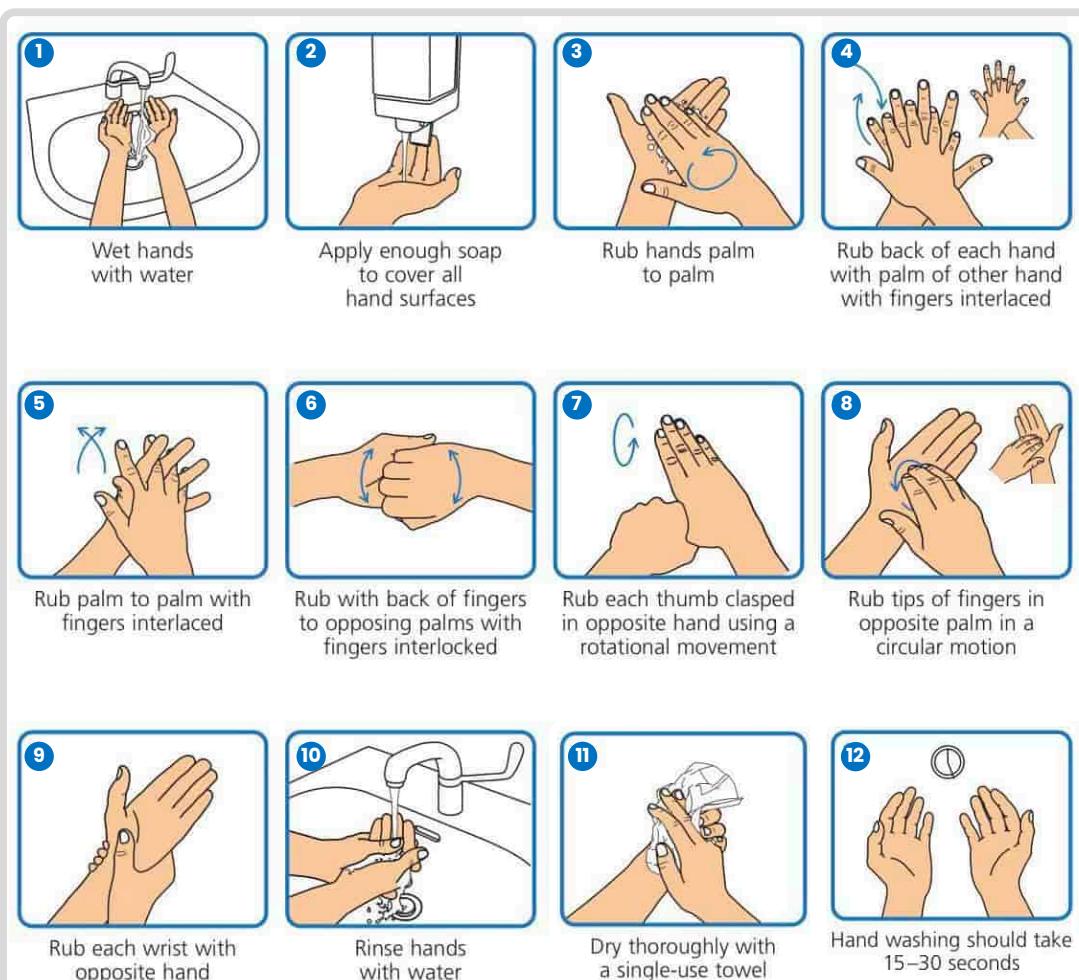
HEALTH & SAFETY PRECAUTIONS

Use standard precautions when handling specimens containing blood or other potentially infectious material. In handling human specimens, the goal is to protect our valuable employees from exposure to blood and to other potentially infectious body fluids.

The following Safety Protocol MUST be followed at all times during specimen collection:

- Observe standard safety precautions and all applicable isolation procedures.
- PPE's must be worn at all times.
- Wash hands in running water with an appropriate hand-washing product. Refer below for detailed instructions on hand washing technique with soap and water.
- If hands are not visibly contaminated a commercial foaming hand wash product may be used before and after each patient collection.
- Gloves are to be worn during all phlebotomies, and changed between patient collections.
- Palpation of the phlebotomy site may be performed without gloves providing the skin is not broken.
- A lab coat or gown must be worn during blood collection procedures.
- Needles and hubs are single use and are disposed of in an appropriate 'sharps' container as one unit.
- Needles are never recapped, removed, broken, or bent after the phlebotomy procedure.
- Gloves are to be discarded in the appropriate container immediately after the phlebotomy procedure.
- All other items used for the procedure must be disposed of according to proper biohazardous waste disposal policy.
- Work areas / contaminated surfaces with potentially infectious material must be disinfected immediately with an appropriate disinfectant such as a 10% dilution of household bleach (0.1% hypochlorite at final concentration).

Hand washing technique with soap & water



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Adapted from World Health Organization Guidelines on Hand Hygiene in Health Care

Exposure Control Plan

Each exposure should be assessed for its potential to transmit HBV, HCV or HIV, based on the route and severity of exposure, and the type of body fluid involved.

To consider an exposure as significant, injured person should come in contact of potentially infectious body fluid in one of the following ways:

- Percutaneous injury: Needle stick or puncture/cut with a sharp object.
- Contact with mucous membranes: Splash to eyes, nose or mouth.
- Contact with nonintact skin
- Human bites resulting in blood exposure to either person involved.

Various body fluids capable of transmitting bloodborne viral pathogens are listed in Table 1 whereas categories of exposure and their severity are listed in Table 2.

If you are exposed to an injury while carrying out specimen collection, do the following immediately:

IMMEDIATE CARE OF THE BODY SITE



Decontaminate the exposed or contaminated site immediately by washing with soap and water for 15 minutes.



Flush eyes with water for 15 minutes either at eye wash station or under the sink. Keep the eyes open and rotate the eyeballs in all directions to remove contamination from around the eyes.



If blood or body fluids splashed into your nose, mouth or skin, flush with water for atleast 15 minutes.

Incident Reporting for needle stick injury



IMMEDIATELY

Provide immediate care to the exposure site.

- ✓ Remove Gloves
- ✓ Wash the exposed area with soap and water
- ✓ Wash mucous membrane or eyes with copious amount of water

DO's

- ✗ Do not panic
- ✗ Do not put the pricked finger in mouth
- ✗ Do not squeeze the wound to bleed it
- ✗ Do not Iodine, antiseptic, chlorine etc. on the wound

DON'T's



AS SOON AS POSSIBLE

Evaluate Exposure and Report

- Seek medical care to determine risk associated with exposure.
- Report blood and body fluid exposure immediately as it poses a risk of infection transmission.
- Reporting as soon as possible will assist obtaining a test from the source.
- Remember to complete an incident report



WITHIN 24 HOURS

- Consult inhouse doctor immediately
- Evaluate significance of exposure (Refer below table - significance of exposure)
- Initiate Post-exposure Prophylaxis (PEP) if the exposure is deemed significant



FOLLOW UP

Perform follow-up testing and get counseling

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Body Fluids Capable Of Transmitting Bloodborne Viral Pathogens

Potentially Infectious Body Fluids	Non-Infectious Body Fluids (Unless Evidently Contaminated with Blood)
• Blood, Serum, Plasma	• Feces
• Amniotic, Pleural, Peritoneal, Pericardial, Synovial & Cerebrospinal fluids	• Nasal Secretions
• Semen & Vaginal Fluids	• Sputum
• Saliva (If its is contaminated with blood & for HBV even if it is not contaminated)	• Tears
• Cultures or HIV, HBV or HCV in special labs, or pathology specimens that contain concentrated viruses	• Urine & Vomit

Significance of exposure

Category	Definition and example
Insignificant exposure	Contact with intact skin.
Mild exposure	Contact with nonintact skin or mucous membrane with small volumes of blood. E.g., superficial skin abrasion with thin bore needles used for subcutaneous injections or contact with eyes or mucous membranes.
Moderate exposure	Percutaneous superficial exposure with solid needle. Contact with nonintact skin or mucous membrane with large volumes of blood. E.g., needle stick injury or cut penetrating gloved finger.
Severe exposure	Percutaneous deep exposure with large volume of blood, e.g., <ul style="list-style-type: none"> • NSI with > 18-gauge needle contaminated with blood • Transfusion of significant amount of contaminated blood • Injury with used intravenous/intra-arterial cannulas • Formation of a deep and painful wound
	<p>Wearing gloves serves as one of the protective factors against such injuries.</p> <p>Note: For NSI with sharps/needles contaminated for more than 48 hour prior to exposure, risk becomes negligible for HIV, and remains for HBV.</p>

(Source: CDC 2005; NACo 2007 Guidelines)

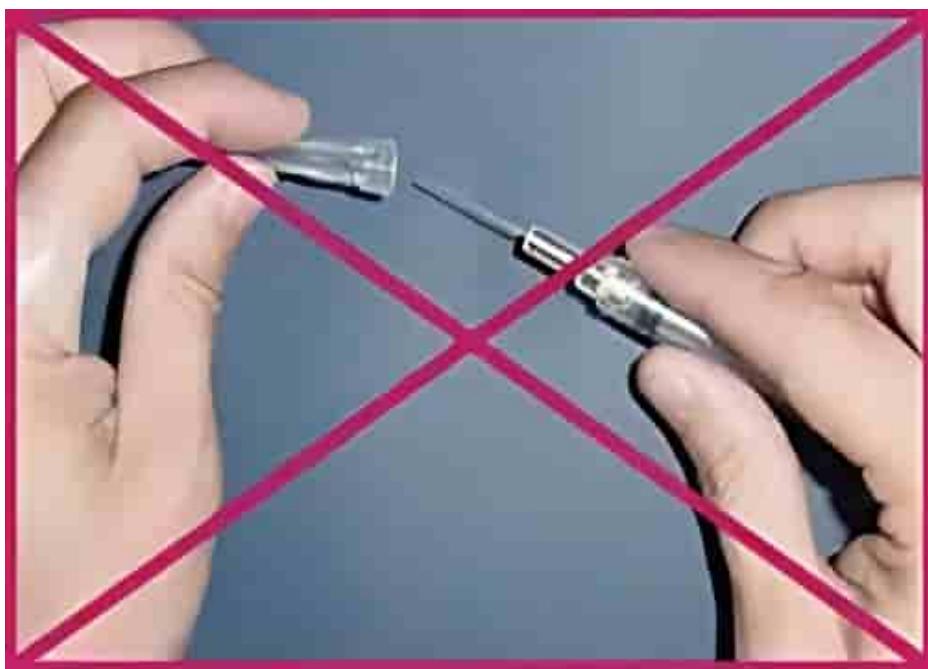
Abbreviations: HIV – human immunodeficiency virus; HBV – hepatitis B virus; NSI – needle-stick injury.

Prevention from needle stick injury

Prevention is the Best Strategy

Prevention of occupational exposures should be the primary strategy. All TRUSTlab Health Care Workers should practice Universal precautions, which include:

- Washing hands before and after care of each patient.
- Appropriate use of protective equipment and devices—gloves, masks, gowns, boots etc.
- Any arterial or venous cannulation should not be performed without wearing protective gloves.
- Needles and sharps should be used with caution:
 - Sharp disposal container must be on the procedure trolley itself.
 - Sharps should be disposed in puncture proof containers immediately after use.
 - Needles should never be recapped.



Needle recapping is a medical sin

Vaccination

Among the three potential bloodborne infections, vaccination is available only for HBV. All TRUSTlab health care workers should receive HBV vaccine and response to vaccination should be documented by measuring anti-HBs titers one month after completion of vaccination.

Periodic Training

All TRUSTlab health care workers should undergo periodic training to minimize occupational exposures. All TRUSTlab health care workers should have access to postexposure prophylaxis (PEP) in case of an inadvertent occupational exposure.

PATIENT PREPARATION

The accuracy and reliability of clinical laboratory results depend directly on the quality of the specimen submitted for analysis. Specimens must be collected by a qualified phlebotomist with an Authorized to Bleeding Certificate that is issued by TRUSTlab. Most of the sample collections can be carried out at the patient's home through our home collection services. For a few specialty sample collections, the patient is required to visit a hospital or at a TRUSTlab Diagnostics Health Service Center (HSC) or a TRUSTlab Authorized sample collection center. Specific specimen requirements for each test are outlined in our online Directory of Services (DoS). For any uncertainties, please contact the laboratory for clarification before collecting specimens.

Submitting an adequate specimen volume is essential for analysis. The volume specified in this directory is sufficient for initial testing as well as any necessary confirmatory tests. If the specimen quantity is insufficient, the laboratory report will indicate "QNS" (Quantity Not Sufficient), and the required tests may not be performed. Adhering to these guidelines ensures the integrity of test results.

Many tests require specific patient preparation (e.g., fasting, diets, urinary voidings).

Fasting Requirements

To ensure accurate test results, the following guidelines apply to fasting specimens for tests performed on serum, plasma, or whole blood:

Definition of Fasting

*Fasting is defined as **no consumption of food or beverages (except water)** for a period of **9–12 hours** prior to specimen collection.*

Why Fasting is Preferred

*Non-fasting specimens may contain **fat particles** (lipemia) that can interfere with many analytical procedures, leading to inaccurate test results.*

Patient Preparation During Fasting

- Strenuous activity should be avoided during the fasting period as it may impact test results.
- Adequate water intake is encouraged to prevent dehydration, which can influence test outcomes.
- Avoid activities or conditions that could lead to acute inflammation or other physiological changes that may alter test interpretations.

GLUCOSE TOLERANCE TEST

Some diagnostic tests require the patient to ingest a specific substance, such as the Glucose Tolerance Test (GTT), which is commonly used to assess the body's ability to metabolize glucose. For the standard GTT, adults ingest 75 g of a glucose solution, while children consume a glucose amount proportional to their body weight (1.75 grams of glucose per kilogram, up to a maximum of 75 g). Blood specimens are collected before ingestion (as a fasting baseline) and at various intervals after consumption to measure the concentration of glucose in plasma or serum. It is important for patients to follow specific instructions regarding ingestion and timing to ensure accurate results.

- The patient should be on a normal diet containing normal daily requirement of carbohydrates (i.e approximately 150 gm per day) at least for 3 days prior to the test.
- Patient must come to the laboratory after overnight fasting.
- The individual should not eat food, drink tea or coffee, exercise vigorously or smoke cigarettes before and during the entire test period.
- Since 2 [TWO] blood specimens have to be collected; one at FASTING, and the second one at 2 hours after 75 gms Anhydrous Glucose- water, the patient has to be made aware of the waiting period of 1½ - 2 hrs at the Collection Centre.
- NOTE: 75 GM OF ANHYDROUS GLUCOSE TO BE TAKEN ORALLY IMMEDIATELY AFTER GIVING FASTING BLOOD GLUCOSE. THE NEXT BLOOD SAMPLES TO BE COLLECTED AT 2 HRS AFTER INGESTION OF GLUCOSE

GESTATIONAL DIABETES SCREENING: GLUCOSE CHALLENGE TEST

A sample should be drawn 1 [ONE] hour after a 50 gram glucose drink is consumed by the patient/customer.

Glucose Level	Indication
Less than 140* mg/dL	Normal Screen
140* mg/dL and over	Abnormal, needs OGTT

Some use a cutoff of 130 mg/dL because that identifies 90% of women with gestational diabetes, compared to 80% identified using the threshold of 140 mg/dL.

GESTATIONAL DIABETES SCREENING : OGTT

Timed collections:

For some hormonal studies, specimen is collected at specific timings. For S. cortisol: Blood specimen is collected at 8 AM, 4 PM. & 8 PM The timing of blood collection has clinical significance, hence must be clearly specified on the primary collection tube. For Dexamethasone Suppression Test: Collect blood sample for Cortisol at 8 hours post 1 mg dexamethasone given at midnight. For Adrenocorticotrophic Hormone Stimulation Test: Collect 3 set blood samples for Cortisone before administrating Co-syntropin I.V./I.M., then at 30minutes and again at 60 minutes post 25 units [0.25 mg] of parenteral Co-syntropin I.V./I.M.

DUAL MARKER

3 ml serum, LMP date, USG report with Nuchal Translucency (NT) Thickness measurement: [recommended on the same date of blood sample drawn] / 6 days prior to sample date. Diabetic status, Smoking and previous history. Weight, Valid between 9.6-14.6 weeks of gestation. It is also known as 1st Trimester Risk Assessment.

TRIPLE MARKER

3 ml serum, LMP date, Diabetic status, Smoking and previous history. Weight, Valid between 14.6–21.6 weeks of gestation. It is also known as 2nd Trimester Risk Assessment.

QUADRUPLE MARKER

3 ml serum, LMP date, Diabetic status, Smoking and previous history. Weight, Valid between 14.6–21.6 weeks of gestation. It is also known as 2nd Trimester Risk Assessment.

Lipid Profile

Customers/patients should be made aware that specimen is collected after an overnight fasting for 10 to 12 hrs. Where possible, they also should be made aware that normal diet should be followed for at least 1 week before the test.

PATIENT PREPARATION FOR HIV TEST

As per NACO Guidelines, HIV sample for testing is collected after obtaining consent in a written format; precounselling and postcounselling for testing is done by treating physician. However, patients are at liberty to ask for precounselling and postcounselling session by trained lab staff. All abnormal results are released after taking proper clinical history or interacting with treating doctor. One to one postcounselling session is given by lab staff when the client can attend to the lab. Proper confidentiality is maintained during the counseling.

SPECIMEN COLLECTION MATERIAL

All specimen collection devices supplied by TRUSTlab are intended exclusively for the collection of specimens to be processed by TRUSTlab.

The following blood collection materials are required for collecting routine specimens:

- **Safety needles / Butterfly needles:** The needles have a bevel which is the slanted opening at the end of the needle and the length of the shaft ranges from 1 to 1 ½ inches. There is a threaded hub. This screws into the needle holder. The needles have a rubber sheath which makes it possible to draw several tubes of blood by preventing leakage of blood as tubes are changed.
- **Vacutainer tube holder:** The holder for vacuum blood collection is a plastic sleeve into which the phlebotomist screws the double-pointed needle.
- **Vacuum collection tubes:** Vacuum collection tubes are glass or plastic tubes sealed with a partial vacuum inside by rubber stoppers. The air pressure inside the tube is negative, less than the normal environment. After inserting the long end of the needle into the vein, the phlebotomist pushes the tube into the holder so that the short end needle pierces the stopper. The difference in pressure between the inside of the tube and the vein causes blood to fill the tube.
- Syringes.
- Antiseptic, individually packed 70% isopropyl alcohol wipes.
- Disinfectant / Germicidal wipes.
- Adhesive band-aid.
- Cotton roll.
- Single useTourniquet.
- Disposable gloves.
- Barcode labels.
- Test Requisition Forms.

SUPPLIES

We provide essential supplies needed for collecting and submitting specimens for analysis in our laboratories. The type and quantity of supplies must correspond to the number of specimens you submit for testing.

For TRUSTlab Home Collection Services, the above-mentioned specimen collection materials are provided in a custom-designed and in-house packaged Blood Collection Kit.



Prohibited Uses

These materials must not be used for:

- Storage or disposal of biological materials, including but not limited to sharp instruments.
- Any activity not directly related to the collection of specimens for processing by TRUSTlab.

Compliance with this policy ensures the safe and intended use of specimen collection devices and supports adherence to relevant safety and quality standards. Non-compliance may lead to appropriate actions as deemed necessary by TRUSTlab.

Note: For ordering of supplies, please contact our Customer Service Department or use the ticketing system in the IT DOSE software.

ORDER OF DRAW

First**Last**

Order	Cap Colour	Tube Type	Number of Inversions	Draw Volume
	-	Blood Cultures	-	-
	-	Blood Cultures	-	-
	Light Blue	Sodium Citrate	3-4	2.7 mL
	Red	Serum	5	6 mL
	Gold	Serum Gel	5	5 mL
	Green	Lithium Heparin	8-10	6 mL
	Lavender	EDTA	8-10	4 mL
	Pink	Crossmatch	-	6 mL
	Royal Blue	Trace Elements	8-10	6 mL
	Gray	Fluoride Oxalate	8-10	2 mL

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Specimen collection tubes other than blood

Container	Volume	Preservative	Section	Comments
CSF	3 ml	No	Microbiology, Core lab (Hem, Chem), Flowcytometry	Sterile
Urine Collection Cup-Plastic	120 ml	No	Microbiology	Sterile
Urinalysis tube: BD vacutainer, yellow top	7-8 ml	No preservative	Microbiology	Maintains urine for up to 72 hours at room temperature, prevent bacterial growth, leak proof
Sputum container	60 ml	No	Microbiology	Sterile
Petri dishes for mycology specimens		No	Microbiology	
Urine Culture tube: BD vacutainer plus C&S boric acid based with light gray stopper	4 ml	Lyophilized preservative: Boric acid 2.63 mg/ml Sodium Borate 2.08 mg/ml Sodium formate 1.65 mg/ml	Microbiology	Maintains urine for up to 48 hours at room temperature. Help to prevent overgrowth without toxicity to existing pathogens
Amies Media swab tube, plastic with cap	Swabs in packs for throat, wound, urogenital specimens	Amies modified stuards media swabs with Charcoal	Microbiology	For both aerobic and anaerobic bacteria
AP specimen collection containers (routine)	60 ml, 90 ml, 125 ml, 250 ml, 500 ml, 1L, 5 L	10% formaldehyde	Anatomic Pathology	Tissue Fixative. Ratio of specimen to tissue 1:10
AP specimen collection containers (Frozen section)	60 ml, 90 ml, 125 ml, 250 ml, 500 ml, 1L, 5 L	No additive	Anatomic Pathology	For frozen sections only
Cytopathology specimen collection container	Ranging from 0.5 ml to 2L	No additive	Cytopathology	

SPECIMEN COLLECTION PROCEDURE

ADULTS



1.

Introduction & Explanation of the specimen collection procedure to the patient /customer.

In a clear and professional voice, introduce yourself to the customer by stating your full name and the company you represent.

For example: "Hello Sir/madam, my name is Vikram, and I am with TRUSTlab Diagnostics." I'm going to explain the process so you know exactly what to expect. If you have any questions or concerns at any point, feel free to let me know..."

Review the patient/customer's order

2.

- For Home Collection: Verify the requisition is stamped with the patient/customer's details and confirm the patient/customer's identity by asking them to provide their full name (first name, last name, and middle initial) and complete date of birth (day, month, and year). All the details on the test requisition form must be filled in for transparency, accuracy and traceability.
- For Collection Center: Prepare a test requisition form by filling in all the fields on the test requisition form, including the tests requested. While filling in the details, request the customer for an ID proof so that the details are accurate.
- Ensure that the customer's phone number is accurately recorded on the test requisition form. A valid phone number is mandatory for the patient/customer to access their test history through the TRUSTlife App or the TRUSTlab website.
- Ensure that the requisition form and barcode labels match the patient/customer's identification exactly, with no discrepancies.
- If the details on the test requisition form and the patient/customer ID do not match, inform the patient/customer and call TRUSTlab's Customer Service Department to reconfirm.



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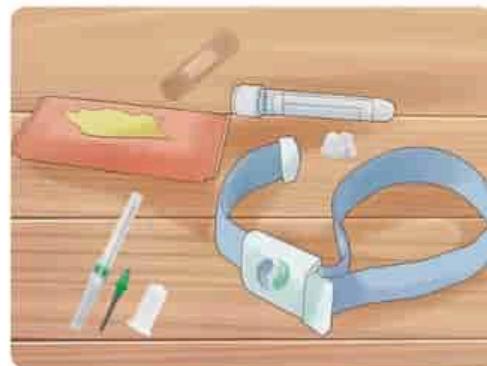
**3.****Wash and sanitize your hands**

Thoroughly wash your hands with soap and water, ensuring you clean all surfaces, then sanitize them using an appropriate hand sanitizer. Once sanitized, allow your hands to air dry or use a clean paper towel before putting on gloves.

Assemble your supplies**4.**

You should have in front of you: a Vacutainer tube holder, needles, specimen collection tubes, a tourniquet, cotton balls, bandage or medical adhesive tape, and Antiseptic, individually packed 70% isopropyl alcohol wipes.

Make sure that your specimen collection tubes have not expired.

**5.****Select the appropriate needle**

The type of needle that you choose will depend on the patient/customer's age, physical characteristics and the amount of blood that you plan to draw. The selection of the needle is based on the gauge number of a needle that indicates the diameter of its lumen. The lumen, also called the bore, is the circular hollow space inside the needle. The higher the gauge, the smaller the lumen. The most frequently used gauges for phlebotomy are 20, 21 and 22.

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**6.**

The chair used for the procedure must have an armrest to support the patient's arm and should not have wheels to ensure stability -

-and safety. Start by disinfecting the chair thoroughly to maintain proper hygiene.

Politely request the patient/customer to sit on the chair and determine which arm to use for the blood draw, either by deciding yourself or allowing the patient/customer to choose.

Position the selected arm on the armrest, ensuring it is fully extended and not bent at the elbow.

If the patient/customer is lying down, provide additional support by placing a clean pillow under their arm to ensure comfort and stability during the procedure.

Tie the Tourniquet

7.

Tie a tourniquet around the patient/customer's arm about 4" to 5" (10cm to 13 cm) above the venipuncture site.

Never leave the tourniquet in place longer than one minute.

**8.**

Ask the patient/customer to gently make a fist to help the veins become more palpable. Avoid instructing the patient/customer to pump their fist, -

-as this can increase blood flow into the vein and cause it to appear larger, potentially affecting the procedure.

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**9.**

Trace the patient/customer's veins with your index finger.

There are different types of veins:

- Superficial veins of the upper limb.
- Median cubital vein - a superficial vein, most commonly used for venipuncture, it lies over the cubital fossa and serves as an anastomosis between the cephalic and basilic veins.
- Cephalic vein - Shown in both forearm and arm, it can be followed proximally where it empties into the axillary vein.
- Basilic vein- Shown in the forearm and arm, it divides to join the brachial vein.
- Usually, an anti cubital vein is chosen. If one is not prominent, choose some other prominent vein for venipuncture. Do not choose a thrombosed vein (a vein feels to be like a cord).
- Tap the vein with your index finger to encourage dilation.

Disinfect the area you plan to puncture using an alcohol wipe

10

Clean the skin in a circular motion, starting from the center of the site and moving outward.

Avoid dragging the wipe over the same area of skin more than once to prevent contamination.

**11.**

Allow the disinfected area to air dry completely for about 30 seconds

This ensures the antiseptic is effective and helps minimize any stinging sensation when the needle is inserted.

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**12.**

Carefully inspect the needle for any defects before use

Ensure the tip is free from obstructions, and without any hooks or irregularities that could restrict blood flow or cause discomfort during the procedure.

Thread the needle into the needle holder

13.

Carefully thread the needle into the holder and use the needle sheath to securely fasten it in place. Ensure the needle shield remains in position and is not removed until ready for use to maintain sterility and safety.

Make sure that your specimen collection tubes have not expired.

**14.**

Gently tap the blood collection tubes

Vacutainers contain additives to dislodge any additives that may have settled on the walls of the tube. This ensures proper mixing and activation of the additives for accurate test results. Avoid shaking the tubes to prevent damage or improper mixing.

**15.**

Carefully insert the blood collection tube onto the holder

ensuring it fits securely. Avoid pushing the tube past the recessed line on the needle holder, as this could release the vacuum and affect the sample collection.

Grasp the patient/customer's arm firmly

Ensure your thumb pulls the skin taut about 1" to 2" (2.5cm to 5cm) below the puncture site. This helps create a stable surface for the needle insertion.

Ensure the patient/customer's arm is positioned slightly downward to prevent blood from flowing backward (reflux) during the procedure.

**16.**

16.

**17.**

Carefully align the needle with the vein

Ensure the bevel (the angled opening) is facing upward. This positioning helps ensure a smoother insertion and minimizes discomfort for the patient/customer.

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**18.**

**Insert the needle smoothly into the vein
at the correct angle**

Once the needle is in position, gently push the collection tube toward the holder until the butt end of the needle pierces the stopper on the tube.

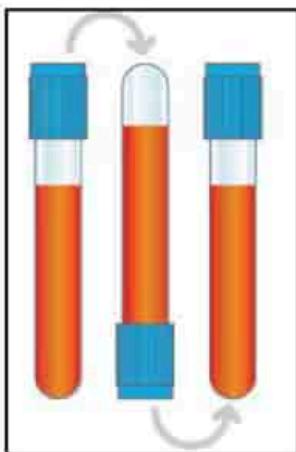
Ensure that the collection tube is positioned below the puncture site to allow the blood to flow into the tube effectively.

**Allow the tube to fill with blood until the
required volume is reached**

Ensure the tube is properly sealed and that the vacuum is maintained throughout the process to ensure accurate sample collection. Fill the remaining tubes according to the required test requisition, ensuring you follow the correct order of draw to prevent cross-contamination between different additives.



Remember to collect tubes without additives before those with additives. Once the blood flow into the tube is adequate, promptly remove and discard the tourniquet to avoid prolonged constriction.

**20.**

While each successive tube is filling, gently invert the previous tube containing additives to mix the sample.

Do not shake the tube, as this can cause hemolysis or damage the sample. Refer to the "ORDER OF DRAW" to ensure you follow the correct number of inversions for each collection tube.

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21. Ask the patient/customer to open their fist gently

This will help relax the veins and allow for easier blood flow after the sample has been collected.

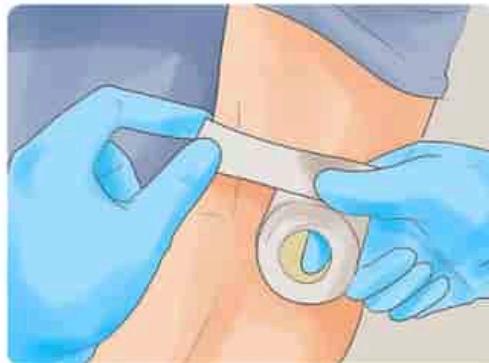
Carefully remove the needle 22.

Immediately place a cotton gauze over the venipuncture site. Apply gentle pressure to help stop the bleeding, ensuring the patient/customer keeps their arm still during this process.



23. Tape a bandage securely over the puncture site

Instruct the patient/customer to keep the gauze in place for at least 30 minutes to ensure proper clotting and prevent any bleeding from restarting.



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24. Discard the needle

Activate the needle's safety feature to cover the needle and prevent accidental needlestick injuries.

Immediately discard the needle into a designated sharps container or a needle cutter device to ensure safe disposal.

Label the vacutainer tubes in the presence of the patient/customer

25.

ensuring the patient/customer's details are clearly written on the tubes. Read the patient/customer's name aloud to confirm the accuracy of the labeling. If necessary, place the labeled specimen vacutainer tubes into a chiller box to maintain the correct temperature during transport.



26.

Clean the surface thoroughly

and wipe the armrest of the chair or the surrounding area with germicidal wipes to maintain hygiene. Discard all used materials in the appropriate waste containers (mobile waste disposal materials for Home Collections), including any used gloves, gauze, and wipes.

Finally, pack away all your materials and equipment in a clean and organized manner.



SPECIMEN COLLECTION FOR PAEDIATRIC AND NEONATAL

Choice of procedure and site

The choice of site and procedure (venous site, finger-prick or heel-prick – also referred to as “capillary sampling” or “skin puncture”) will depend on the volume of blood needed for the procedure and the type of laboratory test to be done. Venepuncture is the method of choice for blood sampling in term neonates; however, it requires an experienced and trained phlebotomist. If a trained phlebotomist is not available, the physician may need to draw the specimen. Patient immobilization is crucial to the safety of the paediatric and neonatal patient undergoing phlebotomy, and to the success of the procedure. A helper is essential for properly immobilizing the patient for venepuncture or finger-prick, as described in Section 6.2.

Venepuncture is the preferred method of blood sampling for term neonates, and causes less pain than heel-pricks.

Equipment and supplies for paediatric patients.

- Use a winged steel needle, preferably 23 or 23 gauge, with an extension tube (a butterfly):
 - avoid gauges of 25 or more because these may be associated with an increased risk of haemolysis;
 - use a butterfly with either a syringe or an evacuated tube with an adaptor; a butterfly can provide easier access and movement, but movement of the attached syringe may make it difficult to draw blood.
- Use a syringe with a barrel volume of 1–5 ml, depending on collection needs; the vacuum produced by drawing using a larger syringe will often collapse the vein.
- When using an evacuated tube, choose one that collects a small volume (1 ml or 5 ml) and has a low vacuum; this helps to avoid collapse of the vein and may decrease haemolysis.
- Where possible, use safety equipment with needle covers or features that minimize blood exposure. Auto-disable (AD) syringes are designed for injection, and are not appropriate for phlebotomy.

Preparation

Ask whether the parent would like to help by holding the child. If the parent wishes to help, provide full instructions on how and where to hold the child; if the parent prefers not to help, ask for assistance from another phlebotomist.

Immobilize the child as described below.

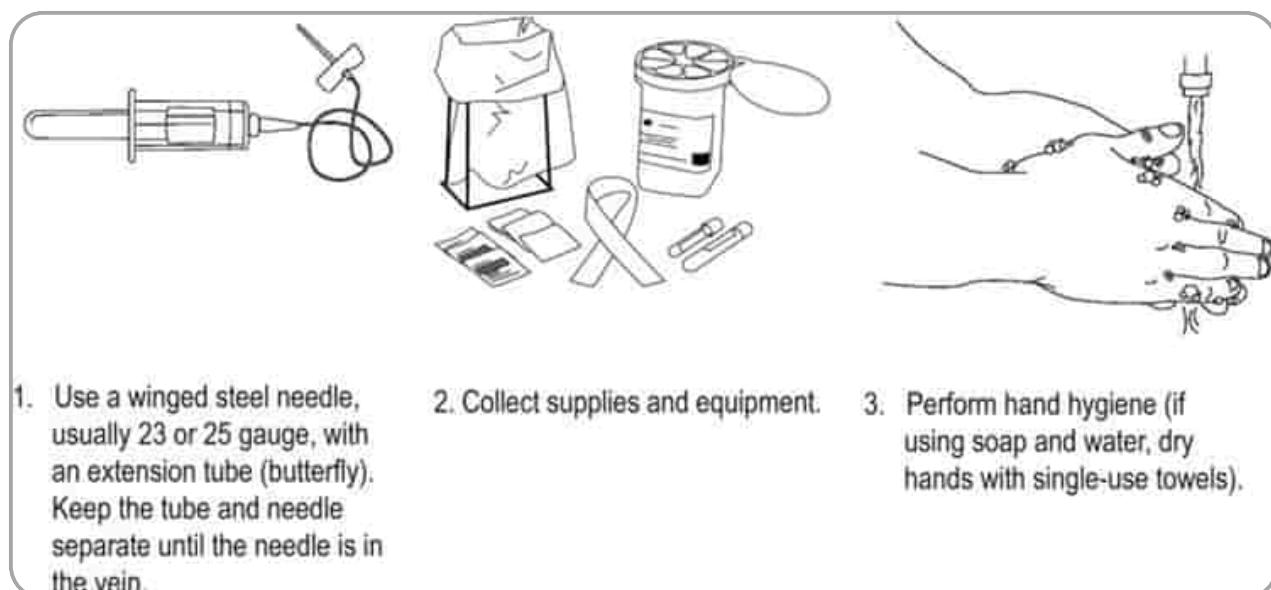
- Designate one phlebotomist as the technician, and another phlebotomist or a parent to immobilize the child.
- Ask the two adults to stand on opposite sides of an examination table.
- Ask the immobilizer to:
 - stretch an arm across the table and place the child on its back, with its head on top of the outstretched arm;
 - pull the child close, as if the person were cradling the child;
 - grasp the child's elbow in the outstretched hand;
 - use their other arm to reach across the child and grasp its wrist in a palm-up position (reaching across the child anchors the child's shoulder, and thus prevents twisting or rocking movements; also, a firm grasp on the wrist effectively provides the phlebotomist with a "tourniquet").

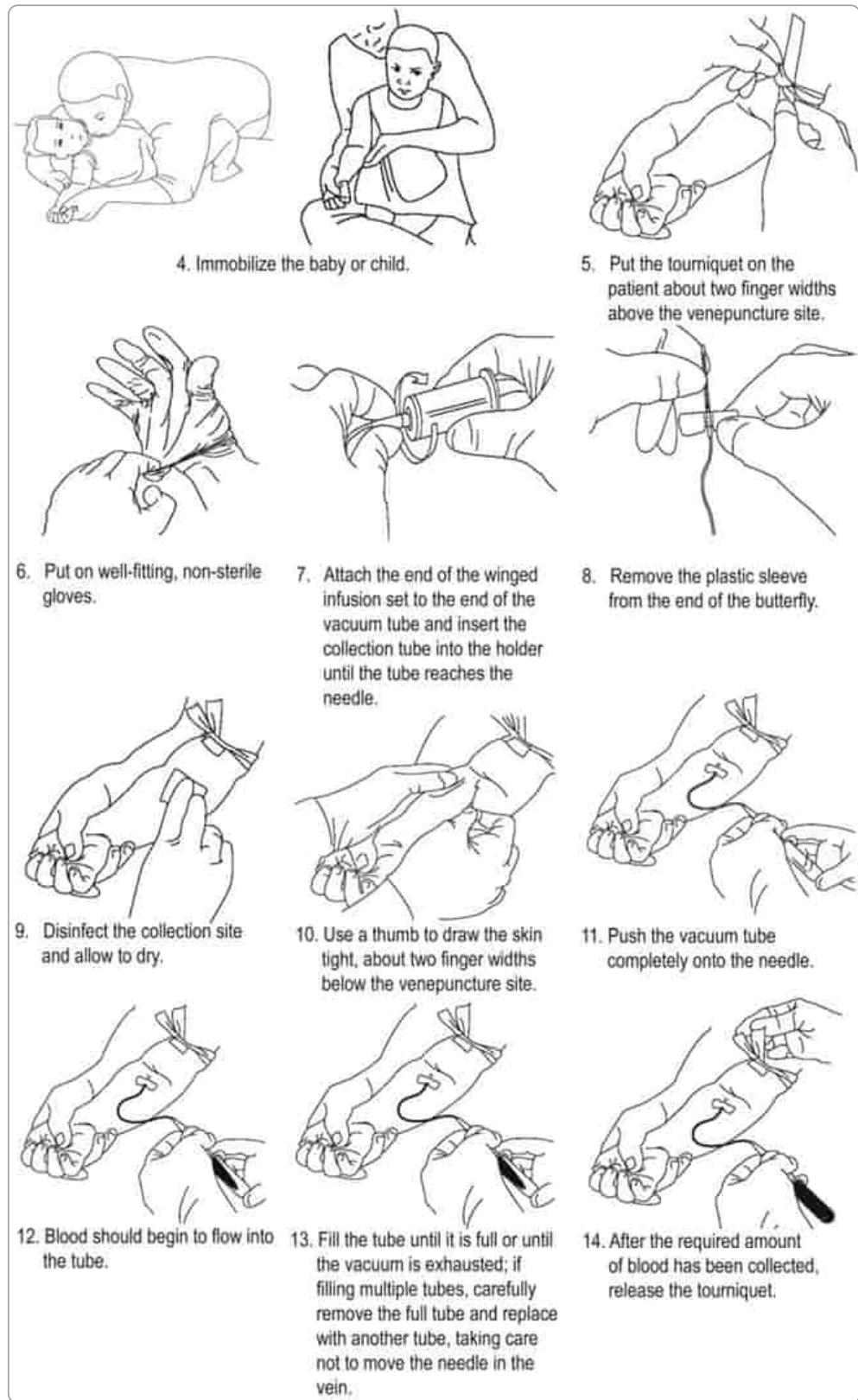
If necessary, take the following steps to improve the ease of venepuncture.

- Ask the parent to rhythmically tighten and release the child's wrist, to ensure that there is an adequate flow of blood.
- Keep the child warm, which may increase the rate of blood flow by as much as sevenfold (65), by removing as few of the child's clothes as possible and, in the case of an infant, by:
 - swaddling in a blanket; and
 - having the parent or caregiver hold the infant, leaving only the extremity of the site of venepuncture exposed.
- Warm the area of puncture with warm cloths to help dilate the blood vessels.
- Use a transilluminator or pocket pen light to display the dorsal hand veins and the veins of the antecubital fossa.

Drawing Blood

- Follow the procedures given in Specimen Collection Procedure for:
 - hand hygiene;
 - advance preparation;
 - patient identification and positioning;
 - skin antisepsis (but DO NOT use chlorhexidine on children under 2 months of age).
- Once the infant or child is immobilized, puncture the skin 3–5 mm distal to (i.e. away from) the vein; this allows good access without pushing the vein away.
- If the needle enters alongside the vein rather than into it, withdraw the needle slightly without removing it completely, and angle it into the vessel.
- Draw blood slowly and steadily.





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15. Place dry gauze over the venepuncture site and slowly withdraw the needle.



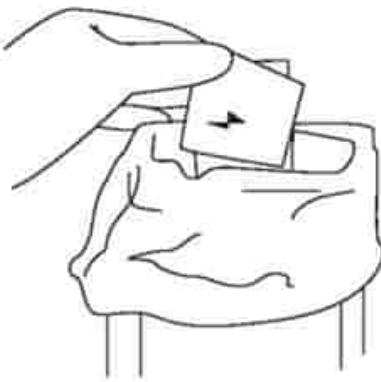
16. Ask the parent to continue applying mild pressure.



17. Remove the butterfly from the vacuum tube holder.



18. Dispose of the butterfly in a sharps container.



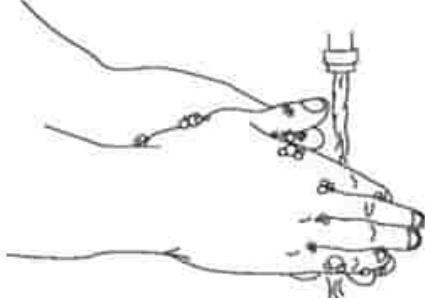
19. Properly dispose of all contaminated supplies.



20. Label the tube with the patient identification number and date.



21. Put an adhesive bandage on the patient if necessary.



22. Remove gloves, dispose of them appropriately and perform hand hygiene (if using soap and water, dry hands with single-use towels).

PEDIATRIC COLLECTION VOLUMES

When infants and children are to be drawn for laboratory testing, consideration should be given to collect the necessary and minimum volume needed for requested tests. To ensure that the circulatory integrity of the younger patient is not compromised, follow the recommended maximum draw volumes in the following table.

Pediatric Weight Chart for Blood Collection

	Maximum <u>mL</u> amount to be drawn in 24 hours	Maximum <u>mL</u> amount to be drawn in 30 days
Kg (Kilograms)	2.5% of total blood volume	5% of total blood volume
1	2.5	6
2	5	10
3	6	12
4	8	15
5	10	20
6	12	24
7	14	28
8	16	32
9	18	36
10	20	40
11 thru 15	22-30	44-60
16 thru 20	32-40	64-80
21 thru 25	42-50	84-100
26 thru 30	52-60	104-120
31 thru 35	62-70	124-140
36 thru 40	72-80	144-160
41 thru 45	82-90	164-180
46 thru 50	92-100	184-200
51 thru 55	102-110	204-220
56 thru 60	112-120	224-240
61 thru 65	122-130	244-260
66 thru 70	132-140	264-280
71 thru 75	142-150	284-300
76 thru 80	152-160	304-350
81 thru 85	162-170	324-340
86 thru 90	172-180	344-360

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Based on blood volume of:

1 to 2 kg	100 mL/kg	(pre-term infant)
>2 kg	80 mL/kg	(term infant - adult)

- 3ml/kg/day maximum recommended blood draw volume.
- Physician approval is required if maximum volume is to be exceeded for a 1 time draw.

Adapted from Children's Hospital in Los Angeles, Baylor College of Medicinein Dallas, TX and Seattle Children's Hospital Laboratory, Seattle, WA.

**CAPILLARY BLOOD (CAPILLARY PUNCTURES,
SKIN PUNCTURES)**

Capillary Blood is preferred for peripheral blood smear and at times can also be used for other haematological examinations.

Procedure:

- Observe standard bio-safety precautions outlined under bio-safety.
- Obtain capillary blood from fingertips or earlobes (adults) or heels (infants)
- Disinfect the puncture site with spirit, dry the site and puncture skin with a sterile disposable lancet no deeper than 2 mm.
- Wipe away the initial drop of blood. Collect subsequent drops in a microtube or prepare a smear directly from a drop of blood.

Precautions:

- Do not squeeze the site to obtain the blood because this alters the blood composition and invalidates the test values.
- Warming the extremities or placing it in a dependent position may facilitate specimen collection.

PRECAUTIONS DURING VENIPUNCTURE

- If there is air leakage around the needle or loss of vacuum in the tube, replace the vacuum tube.
- When there is difficulty in accessing a vein or when a vacuum tube fills too slowly due to a difficult venipuncture, damage to the red blood cells may result. Correct by collecting a fresh tube when blood flow is established or select another puncture site and collect a second specimen.
- Do not remove the needle from the vein with the vacuum tube engaged. This applies both to the last tube collected during a routine venipuncture and to the tube collected during a difficult procedure.
- Premature removal of the tube causes a rush of air to enter the tube, which may result in damage to the red cells.
- Allow the tube to fill completely to ensure the proper ratio of blood to additives.
- Use the collection tubes within their expiration date and store as per the manufacturer's instructions.

If a blood sample is not attainable:

- Reposition the needle.
- Ensure that the collection tube is completely pushed onto the back of the needle in the hub.
- Use another tube as the vacuum may have been lost.
- Loosen the tourniquet.
- Probing is not recommended. In most cases, another puncture in a site below the first site is advised.
- A patient should never be stuck more than twice unsuccessfully by a phlebotomist.

USE OF SYRINGE INSTEAD OF A VACUTAINER METHOD

Syringes should only be used when collecting a specimen from the hand or from individuals with small or fragile veins, where the use of a vacuum blood collection tube may cause the vein to collapse.

For syringe-drawn specimens, transfer blood to the appropriate tubes by pushing the needle immediately through the vacuum tube stopper and allowing the blood to be drawn into the tube until the vacuum is displaced taking caution not to haemolyze the specimen and observing needle safety.

Do not force blood into the tube by pushing the plunger, this can cause haemolysis and may disrupt the ratio of the specimen to the anticoagulant.

AREAS TO BE AVOIDED WHEN CHOOSING BLOOD COLLECTION

- At no time may phlebotomists perform venipuncture on an artery.
- It is not recommended that blood be drawn from the feet.
- Extensive scarring or healed burn areas should be avoided
- Specimens should not be obtained from the arm on the same side as a mastectomy.
- Avoid areas of hematoma.
- If an IV is in place, samples may be obtained below but NEVER above the IV site.
- Do not obtain specimens from an arm having a cannula, fistula, or vascular graft.
- Allow 10-15 minutes after a transfusion is completed before obtaining a blood sample.

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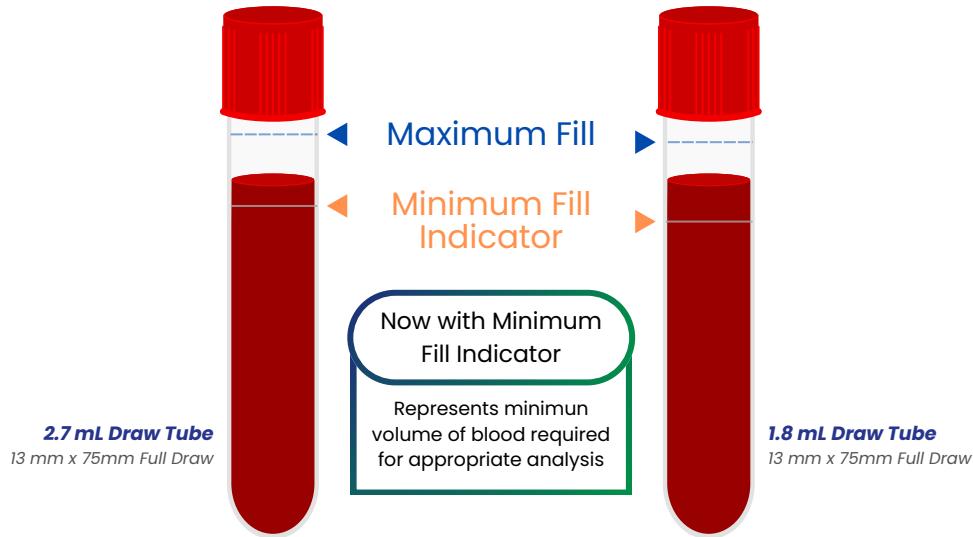
Sources of Error:

- Failure to insert the needle completely into the vein.
- The phlebotomist should feel resistance initially following the insertion of the needle. The resistance is almost immediately followed by a sensation of free or easier movement as the needle enters the vein.
- Puncturing the stopper before entering the vein- If the phlebotomist partially pushes the evacuated tube onto the needle before inserting the needle into the vein, he/she risks puncturing the stopper and releasing the vacuum.
- Not anchoring the vein before inserting the needle. The vein must be held in place for successful needle penetration.
- "Bouncing" the needle on the skin before guiding it into the vein. During venipuncture, the patient should only be stuck once with the needle.
- Not keeping the holder stationary, causing the needle to dislodge from the vein.
- Prolonged tourniquet application (more than 1 minute).

POST PUNCTURE PRECAUTIONS

- Ensure that the bleeding from the puncture site has stopped. If the patient faints, keep the head low with the legs kept at a higher level. Ensure adequate ventilation if thrombosis/hematoma occurs at the puncture site, use thrombophob gel for application at the site.
- It is important to completely fill each tube till the given mark so that the proportion of blood to chemical additive is correct, otherwise, the test results may not be accurate.

VOLUME GUIDE



- Sufficient volume achieved if blood drawn falls above minimum fill indicator. For blood transfer, do not fill above illustrated dashed maximum line.
- Note: The quantity of blood drawn into evacuated tubes varies with altitude, ambient temperature, barometric pressure, tube age, venous pressure and filling technique.
- It is also important to thoroughly mix the blood with the anticoagulant by gentle inversion.

COMPLICATIONS DURING BLOOD COLLECTION

1) Fainting (Syncope)

Signs and symptoms of fainting include: blood draining from the face, rapid breathing, and restless movement. If the client becomes dizzy and faint at the site of blood collection:

- Ask for help to move the client.
- Talk to the client calmly.
- Lie him/her on the patient's examination table.
- Lower his/her head to their knees.
- Apply wet cloth to the back of his/her neck and face.
- Offer him/her a glass of water or orange juice.
- Ask him if he has tendency to faint, if yes lie him down.
- Do not allow the client to leave until he/she recovers.
- If during the procedure client states that he appears to faint, REMOVE THE NEEDLE IMMEDIATELY, lower his head and ask him to breathe slowly and deeply.
- Fill up the incident report.

2) Hematomas

Hematomas occur when the area around the puncture site begins to swell, indicating that blood is leaking into the tissues, which will result in a bruise. Haematomas are due to partial insertion into the vein or piercing through the vein.

If it happens then:

- IMMEDIATELY withdraw the needle.
- Apply pressure for 2 minutes and recheck to ensure bleeding stops.
- Fill up the incident report form

3) In petechiae

In case the client may bleed excessively after blood collection. In such cases, make sure bleeding stops prior to leaving the client.

4) Obesity

Obese clients generally have deeply placed veins and thus, are difficult to visualize or palpate.

5) Excessive Bleeding after venipuncture

Clients on anticoagulants, on aspirin containing medications or having decreased number of platelets, will bleed excessively upon veni-puncture. Do not leave such clients until the bleeding stops.

VARIOUS TYPES OF PRIMARY SAMPLES

<ul style="list-style-type: none"> • Serum 	<ul style="list-style-type: none"> • Sputum
<ul style="list-style-type: none"> • Whole Blood 	<ul style="list-style-type: none"> • Semen
<ul style="list-style-type: none"> • Plasma 	<ul style="list-style-type: none"> • Vaginal Discharge
<ul style="list-style-type: none"> • Body Fluids 	<ul style="list-style-type: none"> • Swabs
<ul style="list-style-type: none"> • Urine 	<ul style="list-style-type: none"> • Human Tissue
<ul style="list-style-type: none"> • Stool 	<ul style="list-style-type: none"> • Aspiration (FNAC)
	<ul style="list-style-type: none"> • Smears (PAP/Cytology)
	<ul style="list-style-type: none"> • Discharge (Nipple/Ear)

Most blood specimens can be obtained using routine phlebotomy techniques; however, there are some exceptions. The use of a tourniquet can cause stress and is not recommended in some cases. Patients should be instructed not to clench their fist(s) prior to or during the phlebotomy procedure as this may alter some of the patient's laboratory results, such as the concentration of potassium in serum. The patient's posture (sitting, standing or supine), or the time of day of phlebotomy can be important factors for some tests (e.g., therapeutic drug monitoring and hormone tests).

SERUM, PLASMA OR WHOLE BLOOD COLLECTION

Draw blood in the color-coded Vacutainer tube indicated in the Order of Draw. For serum or plasma, draw approximately 2 1/2 times the requested volume. For serum, completely fill the Vacutainer and allow the blood to clot in an upright position for at least 30 minutes, but not longer than 1 hour before centrifugation. For plasma and whole blood, completely fill the Vacutainer whenever possible to eliminate dilution from the anticoagulant or preservative and immediately mix the blood by gently and thoroughly inverting the tube 5 to 10 times. Separate plasma by centrifugation.

Transfer the serum, plasma, or whole blood to a plastic transport tube (see the Standard Maximum Blood Draw for Patients Under 14 Years chart for pediatric collections). To prevent injury and exposure to potentially infectious material, do not ship frozen serum, plasma, or whole blood received in glass tubes or SST. The color-coded Vacutainer tubes on the inside cover are recommended unless otherwise indicated in the Order of Draw. Vacutainer tubes are designed for use for both Pediatric and Adult populations.

Handle all biologic samples and blood collection "sharps" (lancets, needles, Luer adapters and blood collection sets) according to the policies and procedures. 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, or other infectious diseases. Use any safety engineered single use needle protector, if the blood collection device provides one Reshelving / recapping of used needles is prohibited. Discard any blood collection "sharps" in biohazard containers approved for their disposal.

WHOLE BLOOD

The most common test using anticoagulated whole blood is the Complete Blood Count (CBC) and blood morphology, which should be collected using a lavender-top (EDTA) plastic vacuum tube. Other tests might require anticoagulants such as heparin (green-top) or sodium citrate (light blue-top) tube. Follow instructions for the individual test.

Collect an adequate volume of blood. Fill the tube to capacity, since partial filling will result in distortions caused by the osmolality of the anticoagulant. Under filled or overfilled blood collection tubes will not be accepted for testing. Immediately mix the blood thoroughly with the additives by gently inverting. Incomplete mixing or delay in mixing after phlebotomy will result in microscopic partial clotting of the sample, which can cause spuriously low platelet counts. Maintain the specimen at room temperature or on cold packs before submitting to our laboratory, unless instructed otherwise by the specimen requirement information in this Directory or by the laboratory. Never freeze whole blood unless it is specifically instructed in the specimen requirement instructions.

If you store cold packs in the freezer, be sure to allow sufficient time for them to warm to refrigerator temperature before placing whole blood specimens near them. To minimize the risk of hemolysis, do not place whole blood specimens in direct contact with cold packs.

PLASMA

Evacuated tubes used to collect plasma specimens contain anticoagulant (e.g., light blue-top tubes contain sodium citrate, green-top tubes contain sodium or lithium heparin, lavender-top tubes contain potassium EDTA).

Consult the individual test specimen requirements to determine the correct additive/tube to use.

Collect a volume of blood that is 2–2½ times the volume of plasma needed for the test. Fill the tube to capacity, since partial filling will result in dilution of the sample.

Do not overfill the tube since it will result in a lower concentration of anticoagulant and activation of clotting. Under filled or overfilled collection tubes may not be acceptable for testing.

- Following the blood collection, immediately mix the tube by inverting the tube gently four (4) times when using light blue-top (sodium citrate) tubes (further inversions might cause activation of clotting factors) and eight (8) times for all others.
- **Centrifuge for at least 10 minutes (horizontal) or 15 minutes (fixed-angle) in a device with a rotor diameter and speed [RPM].**
- Transfer plasma to a properly labeled, clean plastic and tightly capped vial and attach the label from the lower portion of the Test Requisition, if applicable. Do not transfer red cells to the vial. Assure cap is firmly in place to prevent leakage.
- Write "PLASMA" on the plastic screw-cap vial label and on the Test Requisition.

SERUM

For most analyses performed on serum other than therapeutic drug monitoring, we recommend the use of plastic Serum Separator Tubes (SSTs) or plain red-top tubes.

Please check individual specimen requirements for restrictions. SSTs should not be used to collect specimens for drug testing. See Therapeutic Drug Monitoring or Toxicological Analysis in this section.

- Perform venipuncture.
- Collect a volume of blood that is 2–2½ times the volume of serum needed for the test in an appropriate collection tube. Fill the tube to capacity, since partial filling will result in higher serum concentration of tube additives, which are known to alter the results of some tests.
- Immediately mix by inverting the tube gently no less than eight (8) times and no more than ten (10) times. Less than five inversions will result in incomplete clotting and incomplete separation of red cells from serum. Hemolysis of even a small number of red cells remaining above the gel in contact with serum will spuriously elevate results of tests, such as serum potassium and LD.
- Do not remove the stopper at any time. Do not centrifuge immediately after drawing blood. Allow the blood to clot in an upright position for at least 30 minutes, but not longer than 1 hour before centrifugation.
- Centrifuge for at least 10 minutes (horizontal) or 15 minutes (fixed-angle) at 1250 to 1600 RCF (relative centrifuge force) within 1 hour of collection. Spun SST tubes may be submitted without transfer to vials for most room temperature and refrigerated transport temperatures.
- Transfer the clear serum to a properly labeled and tightly capped vial. Attach the label from the lower portion of the Test Requisition, if applicable.
- Write "SERUM" on the plastic capped vial label and on the Test Requisition.

THERAPEUTIC DRUG MONITORING OR TOXICOLOGICAL ANALYSIS

Do not use Serum Separator Tubes (SSTs) for therapeutic drug monitoring, LC/MS/MS or toxicological analysis. The polyester in the separator gel can extract lipophilic substances (most drugs), and can cause a falsely low drug concentration result. Instead, collect the specimen in a red-top tube containing no gel. Collect and process as described above and after centrifugation, transfer the serum with a pipette to a properly labeled plastic vial. Serum should be clear and free of red cells.

Frozen Serum or Plasma Specimens

Serum or plasma specimens need to be frozen when specifically stated. It is essential to freeze the plasma or serum as soon as it is separated from the cells and transferred to a plastic vial. Allow 1-2 mL of space to allow for expansion during freezing. Do not freeze specimens in glass tubes; **always freeze them in plastic vials or tubes—unless instructed otherwise.** Do not freeze plastic Serum Separator Tubes (SSTs).

Lay the tube at a 45° angle to avoid tube breakage caused by expansion during freezing. Hold the specimens before pickup in a freezer or dry ice container. **Do not use frost-free freezers.** The automatic defrost cycle will cause the specimen to partially thaw, and then freeze again. The results of many tests are affected by such freeze-thaw cycles.

Extreme cold may cause ordinary plastic labels to become brittle and detach from the specimen tube. Use clear tape to secure the label to a specimen transport tube.

If more than 1 test is requested on a frozen specimen, split the specimen prior to freezing. Use separate Test Requisitions when submitting more than 1 frozen specimen; frozen and non-frozen specimens must not be submitted on the same Test Requisition. Indicate on the specimen container and on the Test Requisition whether a specimen is plasma or serum.

If more than 1 test is ordered on a single frozen specimen, only 1 of the tests requested will be performed. We will call you to choose which test you want to be performed before testing can proceed.

URINE

Urine Collection

Many urine chemistry tests require a 24-hour collection. Record on the Test Requisition any medications that the patient is receiving. If a preservative is required, it is important that the designated preservative be in the urine collection container at the start of the collection. When the 24-hour urine output is less than 1 liter, 4 grams of boric acid can be used when boric acid is the specified preservative or 10 mL of 6N HCl can be used when HCl is specified. The patient (or responsible individual) should be cautioned that the preservative may be toxic and caustic, and not to spill or discard the preservative.

On the day of the collection, discard the first morning urine void, and begin the collection after this void. Collect all urine for the next 24 hours so that the morning urine void on the second day is the final collection. Measure and record the total urine this volume collected on the Test Requisition and on the urine transport vial (see Pediatric Specimen Tubes below). After mixing the container well, transfer the requested volume into the labeled urine transport vial. Do not send the entire urine collection.

Random (Spot) Urine

The normal composition of urine varies considerably during a 24-hour period. Submit a first morning voided specimen whenever possible because it has a more uniform volume and concentration; its lower pH helps preserve formed elements. To reduce contamination, the specimen submitted should be a "mid-stream" sample. Urine for pregnancy testing should be firstmorning void, or a random specimen with a specific gravity of atleast 1.010. Note the time of collection of the specimen on the Test Requisition and on the container label. For some urine tests, there are dietary restrictions. For others, some drugs must be avoided prior to obtaining the specimen.

Since the concentration of urine varies widely, a convenient way of normalizing test results is to divide the result by the concentration of creatinine in the same aliquot.

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The amount of creatinine excreted daily in the urine is fairly constant (around 1 gram per day) and thus, the amount of creatinine in a random/spot sample is a good estimate of the fraction of the total daily urine volume that the random/spot sample represents.

Type of collection	Collection time	Specimen volume	Preservative	Test	Handling
First morning	First morning collection	2-20 ml	No	Nitrate, protein, pregnancy test, routine screening, microscopy	Refrigerate after 1 hr of collection if not tested
Random (most common)	Any time	2-20 ml	No	Chemical analysis, routine screening, microscopy	Refrigerate after 1 hr of collection if not tested
Mid-stream	Anytime (midstream urine)	2-20 ml	No	Bacterial culture, Chemical screening	Refrigerate after 1 hr of collection if not tested
Post prandial	2 hours after meal	2-20 ml	No	Diabetic monitoring (at point of care)	Refrigerate after 1 hr of collection if not tested

Urinalysis

Specimens must be submitted in a **yellow-top** preservative tube. This tube cannot be used for urine culture. To reduce contamination, the specimen submitted for urinalysis should be a mid-stream sample.

A mid-stream urine is the collection of a sample midway through the urination process. To collect the sample, the patient should begin urinating into the toilet. (A woman should hold apart the genital folds of skin while she urinates.) Midway through urination, a cup should be used to collect the urine without stopping the flow of the urine, until the collection cup is 1/2 to 2/3 full. The patient should then finish urinating into the toilet. The sample should immediately be transferred into a yellow-top urine preservative tube.

24-Hour Urine

Proper collection, preservation and accurate measurement of the volumes of 24-hour urine specimens are essential for accurate test results. Give the patient the clean, labeled container provided by us, and instruct patient not to remove any preservatives (powder, liquid or tablet) that may be in the container. Alert the patient that preservatives are hazardous chemicals and are not to be ingested. Patients should be carefully instructed in the correct collection procedure.

Instruct the patient to carry out steps 1-6 below and return the 24-hour collection to your office for specimen pick-up.

- Unless the physician indicates otherwise, instruct the patient to maintain the usual amount of liquid intake, but to avoid alcoholic beverages.
- During the collection period, place the 24-hour urine container (with appropriate preservatives, if applicable) provided by us in a refrigerator or cool place to prevent growth of micro organisms and possible decomposition of urine constituents.
- Have the patient empty his/her bladder in the morning into the toilet (not to be included in the 24-hour collection). Write the date and time of voiding on the container label.

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- Collect the patient's next voiding and add as soon as possible to the 24-hour container. For traceelement analysis (e.g., heavy metals), the patient should urinate directly into the 24-hour container to avoid contamination of the sample.
- For analyses requiringthe addition of 6N HCl, add the acid at the start of collection. Have the patient collect each void in a smaller container and carefully pour the urine into the 24-hour container to avoid splashing and possible acid burns.
- Add all subsequent voidings to the container as in (4). Thelast sample collected should be the first specimen voided the following morning at the same time as the previous morning's first voiding, which was discarded.

Once the urine collection is complete and received back in your office:

- Mix the contents of the container(s) gently but thoroughly. Examine to help ensure that the contents appear homogeneous.
- Measure and note the total volume of urine. Use the graduated markings on the containerto determine the volume.
- Transfer the required aliquot to the plastic screw-cap specimen container provided by us.
- Record the total 24-hour urine volume on the specimen container and on the Test Requisition before sending to the laboratory.
- If necessary, refrigerate the aliquot untilit can be sent to the laboratory. For frozen specimens, freeze before packing in dry ice for transport. (See Frozen Serum or Plasma Specimens in this section.)
- Ensure the lid is properly tightened to prevent leakage.

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	TIMED URINE COLLECTION		OTHER SPECIMEN / ASPIRATE	
	2 HRS	24 HRS	CATHETER	SUPRA PUBIC
Collection time	2 hrs collection	24 hrs collection	Clamp catheter 15-30 minutes before collection	-
Sample volume	All 2 hrs urine to be saved	All 24 hrs urine to be saved	Variable (2-20ml)	Variable (2-20ml)
Preservative	-	May require preservative (See specific test or contact Laboratory)	-	-
Test(s)	Urobilinogen	Quantitative chemical test	Bacterial culture Foley catheter is not suitable for culture	Bacterial culture
Handling	Test or refrigerate keep in dark bottle	Refrigerate after 1 hr of collection if not tested	Refrigerate after 1 hr of collection if not tested	Refrigerate after 1 hr of collection if not tested

Urine collection for culture & sensitivity

Collect a clean-catch midstream urine sample in a sterile container provided by the Sample Collection Centre.

Urine collection instruction for females

The local area is to be cleaned with soap and water, then again rinsed with water; The labia is to be held apart and patient has to begin voiding; after several ml have passed mid stream urine is to be collected in container; terminal portion is to be discarded again. The containers are to be highly capped and brought to the hospital preferably within an hour.

Urine collection instruction for males

The glans is to be cleaned with soap and water, then reused with water; foreskin retracted, after several mL have passed, collect midstream urine; rest same as above (for females) catheter samples (simple rubber catheter, Foley's catheter) and supra-pubic aspirate for urine culture are to be collected by clinicians.

Patient Preparation:

Instruct the procedures for the patient Specimen collection

- Collection of midstream urine for bacterial investigation:
- Patient not needing assistance:
- Give the patient a suitable container.
- Instruct the patient before the collection, preferably with illustration.
- Tell the patient not to touch the inside or rim of the container.

Male:

- If not circumcised, draw back the foreskin.
- Begin to urinate, but pass the first portion into the toilet.
- Collect the mid-portion of urine into the container, and pass the excess into the toilet.

Female:

- Squat over the toilet and separate the labia with one hand.
- Void the first portion of urine into the toilet.
- Collect the mid-portion of urine into the container and pass the excess into the toilet.

Infants:

Have ready: Clean, preferably sterile container of appropriate size or a plastic bag, cotton wool or gauze pads, luke warm soapy water.

- Clean the external genitals.
- Give the child as much liquid as possible just prior to the collection.
- Seat the child on the lap of the mother, nurse or ward attendant.
- Collect as much urine as possible in the container or plastic bag when the child urinates.

Note: First morning specimens yield highest bacterial counts from overnight incubation in the bladder, and are the best specimens. Colony count interpretation standards are based on controlled studies from first early morning collections. Forced fluids or random specimens dilute the urine and may cause reduced colony counts. Hair from perineum will contaminate the specimen. The stream from a male may be contaminated by bacteria from beneath the prepuce. Bacteria from vaginal secretions, vulva or distal urethra may contaminate transport. Organisms from hands or clothing might contaminate. Receptacle must be sterile.

Urine sample for AFB Screening

Mid stream urine sample is collected, similar to that of a culture specimen, but the sample is collected in five 50 ml sterile containers, 3/4th filled or in a single 200ml volume sterile container.

STOOL

Stool Collection for Chemistry Tests

- Collect timed specimens in a pre-weighed, well-sealed container (available from TRUSTlab).
- At the end of the collection period, determine weight of the total sample.
- Mix contents of timed sample well to obtain a homogeneous mixture.
- Remove the required aliquot to a screw-cap plastic container and seal well.
- Record the total weight and collection time of the sample on both the sample container and the Test Requisition.

Do not send the entire collection unless instructions for a specific test indicate otherwise.

SEmen

Samples to be collected in sterile containers. Samples to be sent immediately after the collection. Avoid bacterial contamination with stool/urine / water / soil/exposure to prolonged sunlight. Samples preferable to be brought to the laboratory within 1 hour if collected outside. Sample collection inside the laboratory is suggested.

Patient should maintain minimum 3 days abstinence, Wash genital area using WATER ONLY. Do not use soap on the genital area prior to collection. Soap may kill the sperm. Remove cap from the specimen container immediately prior to collection. Avoid touching the inside of the container or the inside of the container lid.

Obtain the semen sample by masturbation only. Do not use saliva as a lubricant. The only acceptable lubricant is liquid glycerin. To avoid contamination, do not open the container until just prior to ejaculation. Ejaculate directly into the specimen container. (The penis should not touch the inside of the container). Collect the entire ejaculate. Replace the container lid as soon as specimen is collected to avoid contamination. Submit the specimen to the laboratory. Note: Semen collected in a condom or by coitus interruptus is NOT ACCEPTABLE for Evaluation.

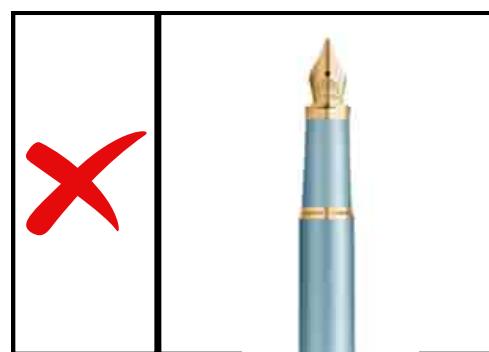
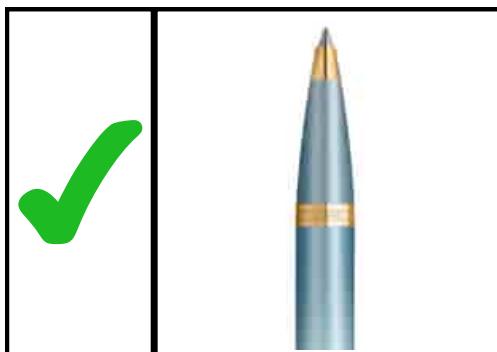
LABELING OF SPECIMENS

All specimens must have a securely affixed label with the pre-printed TRUSTlab barcodes at the time of collection with at least 2 patient identifier that must appear on the test requisition form and match with the specimen label information affixed on the sample transportation media (vial/container).

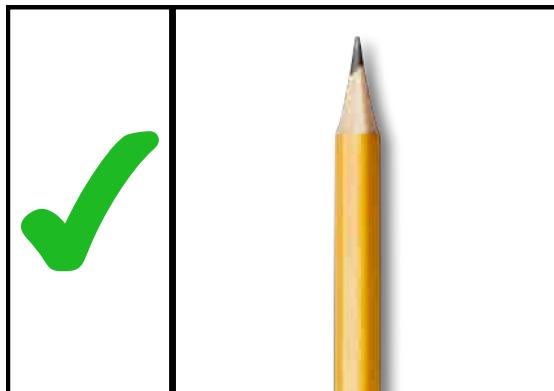
1. The patient's name & date of birth / age is written on the top of the barcode.



-  For labeling specimens, if the label is handwritten, always use a ballpoint pen. Do not use a felt tip pen.



-  For glass slides, label the frosted end using a pencil.



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When using one of our pre-printed or electronically generated barcode labels, the label should be placed lengthwise on the tube to ensure proper alignment and readability.



Poorly written or misaligned labels (where ID cannot be interpreted), will result in affected requests being rejected

These practices help ensure proper identification and minimize the risk of errors in specimen handling and ensure accuracy.

Specimens not conforming to these criteria will be rejected by the Accession Department. Specimens may also be rejected if they have leaked.

It is a requirement and important that the identity of the person who collects each primary specimen and labels them is identified on the test request form along with the date of specimen collection and time.

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TEST REQUISITION

Specimens must be accompanied by a paper requisition, prepared either by hand or printed from an electronic ordering system.



Test Requisition Form

Client Name:	Client Code:																																																										
Patient Name (Block Letters Only).....																																																											
Address																																																											
Mobile No.	Email ID:																																																										
Date of Birth:	Age:	Y:	M:	D:	Gender: Male/Female/Others																																																						
Referring Doctor/Hospital:																																																											
Address & Contact No.																																																											
Sample Drawn Date: Time: AM/PM I.M.P:																																																											
Sample Shipment Date: No. Of Sample Container:																																																											
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 25%;">Test Codes</th> <th style="width: 40%;">Test Description</th> <th style="width: 15%;">Sample Type</th> <th colspan="3" style="width: 20%;">Barcode /SIN No</th> </tr> </thead> <tbody> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> </tbody> </table>						Test Codes	Test Description	Sample Type	Barcode /SIN No																																																		
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Clinical History (Attach relevant clinical details) <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Sample sent @ Temperature:</td> <td style="width: 50%;">Sample Received @ Temperature:</td> </tr> <tr> <td>[18-24°C]</td> <td>[2-8°C]</td> </tr> <tr> <td>[<0°C]</td> <td>[18-24°C]</td> </tr> <tr> <td>[2-8°C]</td> <td>[<0°C]</td> </tr> </table>						Sample sent @ Temperature:	Sample Received @ Temperature:	[18-24°C]	[2-8°C]	[<0°C]	[18-24°C]	[2-8°C]	[<0°C]																																														
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No of Sample containers Received: Receiving Date & Time: AM/PM																																																											
Sample Received By:																																																											
NOTE: Refer TRUSTlab Directory of services www.mytrustlab.com for sample type, collection, reporting time and charges. TRUSTLAB DIAGNOSTICS PVT. LTD. Hyderabad: National Reference Lab, St. No.5, Prakash Nagar, Begumpet, Hyderabad -500016 Vijayawada: Vijnan Skanda Bhawan, Prakash Road, Vijayawada-520002 Bengaluru: Panathur Main Road, Kadubeesahalli, Marathahalli, Bengaluru-560087 Vizag: Opp prudhiana Kalyma Mandapam, Malurripeta, Vizag-530002 Guntur: Old Club Road, Kothaper, Ward No-4, Guntur-522001 TollFree - 1800 599 5656 0 , Home Collection: +91-7475075700, Email: helpdesk@mytrustlab.com																																																											

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Complete all the sections on the requisition including:

- Client Name & Client Code (this is relevant for B2B customers only)
- Adequate patient identification (e.g., name, address, telephone number, medical record number)
- Customer/Patient gender
- Customer/Patient's date of birth, or age
- Name and address of the Doctor ordering the test
- Date and time of specimen collection
- Test(s) requested (refer to online DoS for more test-related information, www.dos.mytrustlab.com)
- Type of specimen
- Relevant clinical information, including any medications that the patient is receiving where appropriate

Warning!

The absence of relevant clinical details may result in delays in specimen processing and, in some cases, lead to specimen rejection.

When ordering tests in a series (e.g., TSH, glucose tolerance, Vitamin B1):

- Use one test requisition for up to 6 tests. In case of ordering more than 6 tests per customer/patient, use a new test requisition form.
- Write/affix the specimen barcode code next to each of the test ordered on the test requisition form.
- Check and note down the temperature while placing the samples in the transportation bag.
- Write the number of specimens on the test requisition.
- Submit all specimens within a series together in one specimen bag.

PACKAGING

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

- a) **A primary receptacle** – e.g. the blood tube or urine container a specimen is collected into;



- b) **Secondary packaging** – e.g. the purpose designed plastic specimen bag.



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.

Warning!

Specimens and test requisition forms / other test supporting paperwork must not be stored in the compartments to prevent contamination in case of leakage from sample containers

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c) **An outer packaging** – e.g. the TRUSTpak transportation bag used to transport specimens to the laboratory by our logistics partners or TRUSTlab logistics team members.



Specimens should be transported to the laboratory using the supplied carriers, e.g. TRUSTpak 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.

Warning!

It is the prime responsibility of the user/sender to collect and package specimens according to the relevant legislation in force and these guidelines. TRUSTlab reserves the right to refuse acceptance of patients' specimens, not packages in accordance with current regulations which pose a hazard to our staff, couriers or other Health Care Workers.

The following are the minimum specimen packaging guidelines that should be followed when submitting specimens using one of our couriers/logistics partners/TRUSTlab logistics team.

- Ensure that all specimen container caps and lids are properly tightened to prevent leakage.
- Double check the specimen container to ensure that the device is not beyond its stated expiration date.
- Although all samples should be treated as HIGH-RISK, (standard precautions), known high-risk specimens should be labelled as 'High Risk'.
- Ensure that ice packs are placed in the TRUSTpak transportation bags to maintain a suitable temperature for the specimens being transported.

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- There should be absorbent material present in the TRUSTpak transportation bags to absorb potential spills and leakages.
- The specimen transport bag has two pouches. Place the specimen container(s) in the front pocket. Insert the requisition into the rear pocket. The requisition bar code must be visible through the bag to allow for proper scanning.
- Frozen specimens should be transported in plastic screw-cap containers only. Frozen specimens must be placed in a separate specimen bag along with a separate test requisition. Frozen specimens cannot be split for other tests. If more than one test is ordered on a single frozen sample, we will call you to authorize which of the tests ordered you want performed before testing can proceed.
- Remove the protective strip and seal the specimen bag. The protective strip must not obstruct the bar code. This will protect the test requisition from leakage and help ensure that the patient information can be entered directly into the laboratory computer by scanning of the bar code.
- If the specimen has been classified as an "infectious substance," transport in a bag designed for such requirements is mandatory. These bags are available from our local laboratory (see the Infectious Substances in the Microbiology section). Please inform TRUSTlab prior to or at the time of our Logistics Representative pickup, so that proper transport arrangements can be made.
- Any updates to these guidelines (or to the specimen transport supplies) will be communicated through a notification from our customer service department.

IRREPLACEABLE SPECIMEN HANDLING

We define an irreplaceable specimen as one which requires an invasive procedure for specimen collection, or one that cannot be recollected. This would include:

- Tissue biopsies or bone marrow submitted for testing (other than routine histopathology)
- Fine needlebiopsies / aspirations
- Body cavity fluids (amniotic, pleural, synovial, ascites)
- Products of conception
- Lavages, washingsor brushings
- Cerebrospinal fluid
- Cord blood
- Kidney stones
- Meconium for drug screening

- Place the collected specimen into the front pocket of the purple bag labeled "Irreplaceable Specimen."
- Fold the requisition and place it into the rear pocket of the bag so the barcode label is viewed in the envelope window; this is critical to enable tracking.
- Your specimen will be tracked accordingly.
- If you have run out of your supply of Irreplaceable Specimen bags and have a specimen to submit to the laboratory, please place the specimen and requisition into a routine bag that is separate from other routine specimens for pickup. Inform the logistics partner/team member that it is an Irreplaceable Specimen, so it may still be tracked as such during transport into the testing laboratory.
- When ordering for refilling packaging materials from TRUSTlab, please request for the Blue bag, as well as, the "Irreplaceable Specimen" labels.

SPECIMEN HANDLING AT THE COLLECTION POINT

Customers are responsible for ensuring that specimens are stored at the required temperatures while awaiting pickup by one of our Logistics Representatives. Proper storage is essential to maintain the integrity of the specimens. Refer to our online **Directory of Services (DoS)** for detailed information on the required specimen storage temperatures to ensure proper handling and preservation.

Frozen Specimens

Frozen specimens must be transported in insulated containers surrounded by an ample amount of dry ice to keep the specimen frozen until it reaches the laboratory. Thawed specimens are unsuitable for analysis. In the event a thawed specimen is received, you will be asked to resubmit an adequate specimen.

If you would like more information about sending specimens to us, please contact our Customer Services Department. Any updates to these guidelines will be communicated through the Laboratory Update and/or by your local Sales Representative.

Needles, Sharps or Medical Waste

Do not send any needles or other sharp or breakable objects. Do not send medical waste as a diagnostic specimen; it may violate the law and create a health hazard. Properly discard used needles or other sharps prior to transport.

Please note that for tests requiring the submission of syringes, the needle must be removed and the syringe capped before sending to the laboratory. Ensure that there is no leakage from or visible contamination outside the specimen container.

Ensuring the Safety of the General Public

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

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The person who has dropped the specimen or noticed that the specimen was leaking should contact one of our trained staff requesting assistance. They should try to ensure that the area is kept clear from all staff, members of the general public, relatives and patients, by enlisting help if possible and available.

Either Biochemistry staff or Haematology staff should be contacted if one of their blood tubes is involved.

The situation should be assessed by our staff for any relevant risks if any associated with the specimen. If no risks as found, our staff will clean up the spillage/leakage according to the procedure in section 29.3 in the Pathology Health & Safety Code of Practice.

If risks are identified, staff should notify the Quality Manager immediately while isolating the area and restricting access.

An incident record should be completed.

SPECIMEN TRANSPORTATION

Responsibility for Transportation

Specimens should be transported to the laboratory as soon as possible after collection, labeling and packing have occurred. Delay could result in deterioration in the specimen which could invalidate the results of any investigations carried out or cause some or all test results to be unavailable.

TRUSTlab logistics team and/or TRUSTlab logistics partners are responsible for providing transportation services between the specimen collection point/center and TRUSTlab laboratory. The TRUSTlab Logistics Department is responsible for training riders and drivers on the handling of pathology specimens, health and safety protocols, timeliness, and safety considerations, including procedures for managing spillages.

Samples are checked physically for the following:

- Correct container
- Labeling
- Clotting status
- Centrifuged status
- Number of vials/ containers
- Temperature condition
- Leakage/ spillage
- Whether accompanied by ice packs if required
- Time fame
- Necessary documents (Test requisition forms, consent form and check list if any)

Monitoring of Transportation of Specimens

Regular audits are carried out of the transport arrangements of pathology specimens to the laboratory from the TRUSTlab audit team and community locations according to documented laboratory procedures.

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 2 and 3 hours) and (ii) transported within a required temperature range.

Where specimens have been collected in a primary care setting and centrifuged, these samples will be stabilized for longer periods and can be transported to the lab the next day.

Sample	Temperature for Transport
Blood for biochemical tests	2 – 8o C
Serum for Immunoassays	2 – 8o C
Slides for microscopy	Ambient
Swabs for viral culture	2 – 8o C*
Histopathology & Cytology	Ambient
Blood for nucleic acid testing	2 – 8o C
Bacteriology specimens (except CSF and blood culture bottles)	2 – 8o C

***VTM (Viral Transportation Medium)** is recommended for transportation of samples for viral culture at 2 to 8 °C. Samples should be transported within 48 hours.

A temperature logger is placed inside the rider's bag and the transportation box during overnight specimen transportation to monitor and record temperature fluctuations. When the samples reach the laboratory, the temperature log is downloaded and reviewed. If the recorded temperature falls outside the required range, the samples may be rejected to ensure the integrity of the results.



Specimen Container Preservatives

Continuous, real-time monitoring of specimens occurs when they are received in the laboratory. This monitoring checks 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 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 a Datix incident 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 on the pathology report.

Reporting of Incidents Relating to Specimen Packing & Transportation

Any adverse incident that occurs in the course of dispatching and transporting pathology specimens to the laboratory must be recorded using the TRUSTlab incident reporting system.

Examples of types of incidents include:

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

Overnight Sample Transportation

- Samples are kept in an upright position in a stand.
- All samples are transported in a Thermocol box with ice packs only on the sides.
- Vacutainers are not to be kept directly on ice packs. CSF sample for culture, PT, APTT is to be sent without an ice pack. DO NOT PLACE TUBES DIRECTLY ON THE ICE PACKS.
- Close the Thermocol box lid and place in the sample transportation box.

SPECIMEN HANDLING AT LABORATORY

- The specimens are accepted at the Accession counter.
- This section is manned by a qualified and trained laboratory staff.
- The designated person checks transport conditions and instructs for corrections if deviations are found.
- Temperature records of the samples are recorded for each packet of samples. If the temperature is beyond 2-80 C, then the samples are rejected or the opinion of the Quality Manager is taken.

SAMPLE ON-HOLD CRITERIA

- **Demographic Issues** - No patient details / Specimens not accompanied by a requisition cannot be accepted. A properly completed request must be submitted / No test name or test code. Sample will be kept on HOLD until Accession receives complete demographic details. If the information is not received before the sample retention period, the sample will be rejected and discarded as per set criteria/procedures.
- **Mismatched specimens and/ or requisitions** - Specimens which are labelled with the wrong patient's name compared to that of the accompanying requisition or with a different patient's ID number, the same criteria as for Unlabeled Specimens apply. The patient/customer is notified and requested to identify the sample. If the information is not received before the sample retention period, the sample will be rejected and discarded as per set criteria/procedures.
- **Unlabeled specimens** - The patient/customer is notified and requested to identify the sample. If the information is not received before the sample retention period, the sample will be rejected and discarded as per set criteria/procedures.
- **Incomplete label** - All specimens must have the patient's required details. The patient/customer will be notified for corrective actions. If the information is not received before the sample retention period, the sample will be rejected and discarded as per set criteria/procedures.

SAMPLE REJECTION CRITERIA

Sometimes tests cannot be performed in the laboratory if samples fall short of the quality, volume or other eligibility criteria. In these cases, the laboratory may need to reject the samples, and not carry out processing.

In situations where the laboratory is able to rectify a situation – and although turnaround times may be affected, it avoids having to arrange for samples to be taken again.

A specimen may be rejected in the following situations:

- **Mismatched specimens and/ or requisitions** – Specimens which are labelled with the wrong patient's name compared to that of the accompanying requisition or with a different patient's ID number, the same criteria as for Unlabeled Specimens apply.
- **Unlabeled specimens** – The patient is notified and requested to identify the sample.
- **Incomplete label** – All specimens must have the patient's required details. Contact the collector to correct any problems.
- **Contaminated specimen** – Sample with the presence of any other foreign material unrelated to surgical/ biopsy procedure/ material likely to affect the test results. Judgment based on the same rests with the reporting faculty.
- **Improper specimen container/ temperature for requested assay** – if the specimen is not in the acceptable container/ not transported at required temperature/ in improper conditions.
- **Insufficient quantity of specimen** – Specimen received in the lab is not enough or not good enough to do the tests requested.
- Samples such as Calculi, Metal Screw, tooth received for Histopathology are rejected.

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- All samples not under scope of testing are rejected.

- **Radioactive labeled specimen** - All biopsy samples labeled as radioactive should be rejected.

- **Broken Slides** - Slides received for review for Cytopathology and histopathology should not be excessively broken. The submitter is notified and requested to submit are-sample.

- **Inadequately prepared slides** - Slides not prepared upto the mark can be rejected and request for a re-sample is raised to the submitter.

- **Request for Cancelation by Clinician** - The sample is rejected if requested by the clinician to do so.

- **Hemolyzed/ lipemic/ clotted sample** - If a sample is of poor quality e.g. hemolysis or clotted, the required tests will not be performed. A repeat sample will be required to complete the request.

- **Aged specimen** - Specimen must be delivered to the lab in a timely manner. Example: All PCR samples must reach the lab within 6 hours from collection time otherwise it will affect the result.

- **Sample leaked during transportation** - Leaking containers cannot be accepted. The submitting lab or physician will be asked to submit a fresh specimen in the proper container, with a new request form if soiled. (Critical specimens, i.e., CSF or body fluids cannot be rejected. Wearing gloves, lab personnel should clean the outside of the container with 10% bleach solution).

- **Inadequately prepared slide** - Slides not prepared up to the mark are rejected and a repeat sample/ slide is requested.

- **Demographic Issues** - No patient details / Specimens not accompanied by a requisition cannot be accepted. A properly completed request must be submitted / No test name or test code.

Quality Assurance Reasons

- Sample contamination (e.g. being in the same bag as a leaking sample).
- Samples are high risk or infectious.
- Samples that are received in expired tubes.

Department-specific Reasons

- Accession department will not accept samples packaged with needles of any kind.
- Haematology cannot accept frozen whole blood for testing.
- Coagulation cannot accept over or under filled samples for testing.
- Coagulation cannot accept previously frozen samples that have thawed in transit.
- Biochemistry cannot accept previously frozen samples that have thawed in transit.
- Biochemistry cannot accept samples that display antibody interference.
- Biochemistry cannot accept samples that have had separation delays/un-centrifuged samples that have been stored in the fridge.
- Biochemistry cannot accept paraprotein resulting in viscous samples.
- Biochemistry cannot accept CSF protein that is blood stained.
- Immunology cannot accept TBQ kits that:
 - Do not contain all of the appropriate tubes.
 - Are incubated for more than the specified 16 hours.
 - Have passed the incubation time period.
 - Are over- or under-filled.
- Microbiology cannot accept samples in non-sterile containers or in formalin.
- Molecular Pathology cannot accept samples for Haemophilia testing without informed consent.
- Cervical Cytology cannot accept over or under filled samples for testing.
- Cervical Cytology cannot accept samples received within three months of the previous test in order to allow epithelial cells to regenerate.
- Urine cytology cannot accept delayed samples unless they have been refrigerated.

Note:

Samples deemed to be PRECIOUS (e.g. CSF, fluid, tissue, bone marrow and paediatric samples) will not be discarded by the laboratory. Results will include a comment relating to the condition of the sample (e.g. sample unlabeled).

If a sample does not meet the required criteria, a request for a repeat sample is made, and the case is treated as a fresh submission. For all rejected samples, a record is maintained indicating "Test Not Reported" along with the specific reasons for rejection, ensuring transparency and proper documentation.

CENTRIFUGATION OF SAMPLE TYPES FOR PROCESSING

The following is a guideline on centrifuging different sample types before they are processed. This guideline is also applicable to TRUSTlab Health Service Centres, where the samples are required to be held for an extended duration before pick-up.

Sample Centrifuge Speed (RPM) and Time		
SAMPLE	SPEED (RPM)	TIME (MINUTES)
SERUM (YELLOW/SST)	3500	10
PLASMA (Blue Top)	3000	15
PLASMA (Grey Top)	2500	5
URINE	2000	5
CSF/BODY FLUID	2000	8
WHOLE BLOOD	3500	8

POST EXAMINATION SPECIMEN STORAGE

Patient samples are to be retained for an appropriate time period so that the investigative process is completed.

- Blood/serum/CSF samples (for biochemical/ serological/ antigen testing) should be stored in tightly capped containers in the refrigerator at temperature 2°–8°C for up to 72 hours (3 days).
- Blood/serum/CSF samples which have been collected for aerobic culture, must be stored either in an incubator or at room temperature. The swab samples may be held at room temperature depending upon the isolate being looked for 24 hours.
- Culture isolates from patient samples- may be preserved for longer periods for research purposes. Otherwise, they are discarded once the patient's report is dispatched after identification and antimicrobial susceptibility testing.
- ATCC control strains/ WHO Reference strains are preserved for as long as they remain pure and uncontaminated.
- The storage of samples should be as per requirement. The outside of the container should be checked for visible contamination which, if present, should be cleaned.
- All the specimen vials must be adequately labeled with the patient's details.
- The blood samples may also be stored at -20° C if they are to be used beyond 7 days.
- The whole blood samples used for cell count testing, are not to be stored for beyond 24 hours at room temperature.
- The stained slides from blood are retained for a period of 24 hours after dispatch of report.
- Samples collected for cytological and histopathological examination- Cytology specimens such as body fluids are stored till 24 hours after dispatch of reports. Stained slides are retained for a period of minimum 5 years. Wet tissues like histopathology specimens are stored for at least 4 weeks after dispatch of reports. Slides and blocks from these tissues are stored for 10 years. This is in accordance with Guidelines issued by College of American Pathologists. Specific storage conditions, detailed protocols and procedures are cited in the specimen collection and handling manual of the Department of Pathology.
- When the samples are stored in a refrigerator the refrigerator should not be used for storing sterile media and kits.

Samples after processing are stored & retained as per the table given below.

S.No		Sample Type	Storage Temp	Storage Period
1	BG	EDTA tubes Hb HPLC samples	2-8°C	7 Days (Hemolyzate)
2	BG	DBS	2-8°C	30 days
3	BG	Urine on FP (DUS)	2-8°C	30 days
4	BG	Plain/ gel tubes for Protein electrophoresis	2-8°C	7 days
5	BG	Plain/ gel tubes for serum amino acids	2-8°C	7 days
6	Biochemistry	Fluoride Tubes	2-8°C	3 days
7	Biochemistry	Plain/ gel tubes	2-8°C	3 days
8	Biochemistry	Citrate Tube (Biochemistry)	2-8°C	72 hours
9	Biochemistry	Urine (Biochemistry)	2-8°C	24 hours
10	Hematology	EDTA tubes Non HPLC samples	2-8°C	72 hours
11	Hematology	Citrate tubes (Hematology)	2-8°C	24 hours
12	Hematology	Urine routine & Microscopy	2-8°C	24 hours
13	Microbiology	Urine C/S	2-8°C	3 days
14	Microbiology	Blood C/S, CSF	RT	10 days
15	Microbiology	Catheter tip, Swab specimens	2-8°C	3 days
16	Microbiology	Sputum C/S	2-8°C	3 days
17	Microbiology	Pus C/S	2-8°C	3 days
18	Microbiology	Stool C/S	2-8°C	3 days
19	Microbiology	Body fluid C/S, except CSF	2-8°C	10 days
20	Molecular biology	Molecular testing HBV/HCV/HIV/COVID-19	-70°C	1 month

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Department Specific Specimen Retention

Clinical Chemistry	Storage Duration	Storage Temperature
Urine	24 hours	2-8°C
Electrophoresis strips and Immuno-fixation plates. If digital images of adequate quality for diagnosis are taken, original preparation may be disposed. Images should be stored as photographic records.	5 years unless digital images are taken). 2 Years	Room temperature
Serum/body fluids	48 hours	2-8°C
CSF	1 month	-70°C

Core Hematology specimens	Storage Duration	Storage Temperature
Whole Blood sample	48 hours	2-8°C
Body fluid	48 hours	2-8°C
CSF specimen	48 hours	2-8°C
Peripheral blood smear/body fluid smears	7 days	Room temperature

Special Hematology specimens	Storage Duration	Storage Temperature
Coagulation plasma (routine)	72 hours	2-8°C
Special coagulation plasma (frozen)	until testing is complete	-50°C
Bone marrow smears (slides)	10 years ideally over life of patient	Room temperature

Flow Cytometry	Storage Duration	Storage Temperature
CSF/body fluids in RPMI	48 hours from collection	2-8°C
Lymph node biopsies in RMPI fluid	48 hours from collection	2-8°C
Tissue specimen (blood/ /bone marrow)	24 hours from collection	Room temperature

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Histopathology Specimen	Storage Duration	Storage Temperature
Gross specimens	2 weeks after final report	Room temperature
Paraffin Blocks	10 years	Room temperature
Frozen tissue for immediate histological assessment (microscopic, residual tissue processed as a normal, fixed specimens once the frozen section is complete)	10 years	Room temperature
Glass slides including control slides	10 years	Room temperature
Internal QC slides	2 years	Room temperature

Cytopathology	Storage Duration	Storage Temperature
Gynecological and non-gynecological slides	5 years	Room temperature
Fine needle aspiration and non-gynecology glass slides	10 years	Room temperature
Non gynecological specimens	48 Hours after reporting	2-8°C
Gynecological specimens	One month after reporting	2-8°C
Cell blocks (paraffin) for fine needle aspirate	10 years	Room temperature
Cell blocks (Gynecological and non - gynecological)	5 years	Room temperature

Specialized Specimen Retention	Storage Duration	Storage Temperature
Serum specimens for HIV confirmatory test	1 year	-30°C
Swabs and urine for Chlamydia OAML, Clearview insert)	2-3 Days (Clear view kit insert)	2-8°C
Urine for Legionella	7 days (Binax kit insert)	2-8°C
Serum taken after needle stick injury or hazardous exposure	1 year	-20°C
Storage of material following analyses of nucleic acids	6 months	2-8°C
Aliquot of send out tests	Until final report received	2-8°C
Immuno-fluorescence slides	48 hours	Room temperature
Proficiency test/material	1 year	-30°C
All serum/plasma specimens	2 weeks -1 month	-20°C (NVRL, NPAAC)

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Cytogenetic Specimen Retention	Storage Duration	Storage Temperature
Fluorochrome stained slides	At the discretion of the lab head	2-8°C
Wet specimen/tissue	Until release of the final report	2-8°C
Permanently stained slides	3 years	Room temperature
Fixed cell pellet	2 weeks after final report	2-8°C

POCT Specimens	Storage Duration	Storage Temperature
Blood gas testing	Immediately discard after testing	N/A
Glucose testing	Immediately discard after testing	N/A

Coagulation Specimen	Storage Duration	Storage Temperature
Whole Blood sample	48 hours	2-8°C
Coagulation plasma (routine)	7 days	2°C
Serum	48 hours	2°C
Blood gas testing	Immediately discard after testing	N/A
Peripheral blood smear	7 days	Room temperature
Blood Group /Antibody Screen /Cross match	7 days	2-8°C

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Microbiology Specimen	Storage Duration	Storage Temperature
Cooked meat	2 weeks	4°C
Environmental Culture	72 hours	4°C
Fungal Culture	4 weeks	4°C
Gram Stain	1 week	Room temperature
Gram Stain for CSF, Body Fluids & Blood Culture	1 week	Room temperature
LIM Broth	72 hours	4°C
Positive Blood Culture Bottles	1 week	Room temperature
Positive Culture Plates	3 Days after final reading	4°C
Semen sample	72 hours	4°C
Stained smear for parasite	1 week	Room temperature
Stool for Adenovirus	72 hours	4°C
Stool for Clostridium difficile toxins A&B	72 hours	4°C
Stool for H.Pylori Antigen	72 hours	4°C
Stool for Rotavirus	72 hours	4°C
Stool O & P	72 hours	4°C
Other Samples O & P	72 hours	4°C
Swab, Respiratory, Stool Culture	72 hours	4°C
Tissue, Frank Pus, Body fluids & CSF	4 weeks	4°C
Trypticase soya broth	72 hours	4°C
Urine for Culture/Urinalysis	72 hours	4°C
Stool for Occult blood	72 hours	4°C

SPECIMEN DISPOSAL

Policy for disposal of samples:

- Beyond the defined retention time, the samples are discarded according to the BMW / Pollution Control Board guidelines.
- Based on their specific tests, TRUSTlab has defined specific sample retention time.
- It is the responsibility of the concerned laboratory technician to discard the samples after the completion of the retention time.
- If indicated the samples may be preserved beyond their defined retention time for academic, research or medico-legal purposes.

HANDLING OF WASTES

- The segregation of wastes is done at the source of generation into yellow, red and blue bags.
- Yellow bags are designated for infectious, non-sharp (pathological and anatomical) waste, including items contaminated with blood or body fluids, soiled bandages, dressings, cotton swabs, blood bags, transfusion sets (non-PVC), body tissues, body parts, and untreated microbiology and biotechnology waste.
- The red bags are meant for infected plastics like oxygen masks, gloves, syringes etc. for sterilization and shredding. These items should not be sent for incineration.
- The red and yellow bags and the sharp cans should be tied, autoclaved & then labeled and handed over to the biomedical waste collection agency. Records of biomedical waste are to be maintained.
- Blue boxes are puncture proof containers for discarding whole and broken glassware. A biohazard symbol is displayed on them.
- Needles used for blood collection are destroyed using a needle destroyer, while other sharp items, such as scalpel blades, are disinfected and safely discarded in designated sharp containers to prevent injury and contamination. The sharps should not be left lying even for a few minutes.

- Pipette tips and other disposables are disinfected either by immersing them in freshly prepared 1% Sodium Hypochlorite solution daily or by autoclaving, ensuring they are rendered non-infectious before disposal.
- Green bags are used for general and household waste, such as food peels and leftover food, while blue bags are designated for dry, non-infectious waste meant for municipal corporation disposal (MCD).
- Polythene bags are routinely placed in waste bins and replaced either daily or when they are 3/4th full, ensuring proper waste containment and minimizing the risk of spillage or contamination.
- Untreated waste should not be stored beyond 48 hrs.

SEGREGATION OF WASTES

- Segregation of biomedical waste at the source significantly reduces the volume of waste requiring special handling and treatment.
- Ensures that medical waste, such as sharps, is kept separate from general municipal waste, preventing contamination, reducing health hazards, and facilitating appropriate handling, treatment, and disposal.
- Prevent the illegal reuse of components such as used syringes, needles, and other plastics, thereby reducing the risk of infections and ensuring public health and safety.

DECONTAMINATION OF WASTES

- The Bio-Medical Waste Management (Amendment) Rules, 2018 require every occupier, defined as a person with administrative control over an institution generating biomedical waste, to pre-treat laboratory waste, microbiological waste, blood samples, tissue waste, processed specimens, and blood bags through disinfection or sterilization on-site. This process must adhere to the guidelines prescribed by the World Health Organization (WHO), including the standards outlined in the WHO Blue Book 2014 on the safe management of healthcare waste. Once pre-treated, the waste must be sent to an authorized Common Bio-Medical Waste Treatment Facility (CBWTF) for final disposal, ensuring compliance with global standards to minimize health risks and environmental hazards.
- Accordingly, all waste generated while collecting and handling samples are disinfected at the site of generation either by treating them with 1% Sodium Hypochlorite solution for 20 minutes or by autoclaving the waste in a designated waste sterilization autoclave at 121°C at 15 lbs pressure for 1 hour.
- Procedures and instructions regarding specific waste handling are detailed in the respective departments.

PACKAGING OF WASTES

- Torn, damaged, or leaking containers must be overpacked by placing them within a second, intact container to prevent spillage, ensure safe handling, and maintain compliance with biomedical waste management protocols.
- Biomedical waste bags are securely tied at the source when they are 3/4th full, ensuring safe handling, minimizing spillage, and preventing overfilling during storage and transportation.

MICROBIOLOGY

General Submission Requirements

Successful isolation of potential pathogens depends upon specimen selection and collection, proper transport and timely delivery to the laboratory.

Whenever possible, specimens should be obtained before antibiotics or other antimicrobial agents have been administered. Please indicate the source of the specimen on the test to better assist in your diagnosis and treatment of the patient.

Not all specimens contain clinically significant pathogens. Organism identification and antimicrobial susceptibility studies will be performed only on appropriate isolates at an additional charge.

- Specimen collection from normally sterile sites requires a needle puncture or a surgical procedure to decrease the chance of contamination. Do not submit syringes with needles attached. If a syringe must be submitted, remove needle, expel air and recap syringe. Tape syringe plunger in position to prevent accidental movement.
- Because specimens are frequently and routinely collected from sites that are not sterile, the quality of culture results is directly dependent on the collection technique for urine, sputum, specimens from the nasopharynx and wounds.
- Specimens from sites such as skin, mucous membranes and the gastrointestinal tract are populated by indigenous microflora. Microbiological tests will be directed at the isolation of specific pathogenic agents.

Temperature

Appropriate storage and transport temperatures for clinical specimens are essential for successful isolation of organisms. If room temperature is required for a specific test, do not place the specimen in an environment where it would be exposed to extremes of heat or cold, so that temperature-sensitive organisms have a better chance of survival. Refrigerated temperature can be maintained using a household or commercial refrigerator (that is not used to store food) or a cooler with cold packs. If refrigeration is requested, do not freeze the specimen.

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Frozen samples may be stored in a household or commercial **non frost-free freezer** (that is not used to store food) at -20 °C until transported to the laboratory. When freezing certain Virology specimens or specimens for C. difficile Toxin analysis, maintain them until transport and during transport at less than or equal to -70 °C, which is best done using dry ice. Cultures for Cytomegalovirus must always be frozen at less than or equal to -70 °C (dry ice) when sent to a referral lab.

FEEDBACK AND COMPLAINT PROCEDURE

- Once the Report is dispatched, the front office executive/Lab people will ask for Feedback about the services of each and every customer.
- In the feedback form mention the patient's Name and Phone number
- If any other suggestion/Complaint is there provision has been given to write in the feedback form.
- A complaint box is also available at reception to put complaints.
- All complaints will be addressed and closed by the Lab Head.

BLOOD FOR CULTURE (AEROBIC / FUNGAL)

- Blood collection is performed only by well-trained experienced phlebotomists (Laboratory technicians/ Doctors).
- Collect blood during fever / spike phase/before administration of antibiotics.
- Collect 7-10 ml in adults, 3-5 ml in children and 1-2 ml in neonates.
- Preferably Collect two specimens from two different sites within an hour of each other or two specimens over 24 hrs.
- Gather material required for collection and biomedical waste disposal, which includes, Identified patient, Tourniquet, Alcohol wipes, Betadine solution, Sterile syringe and needle (21 G preferably) or appropriate evacuated container sets, cotton ball, gloves, alcoholic hand rub solution, container - Automated blood culture bottle (BacT/Alert bottles) brought to room temperature if refrigerated and with the top disinfected with alcohol wipes, pre-labeled, needle and syringe destroyers, sharps can, requisition form, red bag and yellow bag.

Volume of Blood:

Blood culture bottle/Vial	Purpose	Recommended volume of blood to be collected (per bottle)
BacT/ALERT FA Plus Aerobic	For adults	8-10 ml
BacT/ALERT PF Plus Aerobic	For paediatrics	1-3 ml

Procedure:

- Follow instructions as mentioned under collection of blood with the following modifications. Ensure to follow order of draw as mentioned above.
- Pre label the blood culture bottle with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection.

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- Disinfect the site of collection [patient's] with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol]. After use, discard the alcohol swab in the yellow bag.
- Follow this with betadine disinfection in a circular motion beginning from center and moving out. Allow to dry. Discard the cotton swab in yellow bag.
- Take a new sterile needle [preferably 21 G for an adult and 22 G for a child] and syringe in front of the patient. The needle is attached to the syringe.
- Collect adequate volume
- Transfer the blood gently and aseptically into the blood culture bottle along the wall without squirting. Mix the contents well by placing on a horizontal surface.
- Send the specimen immediately to laboratory.

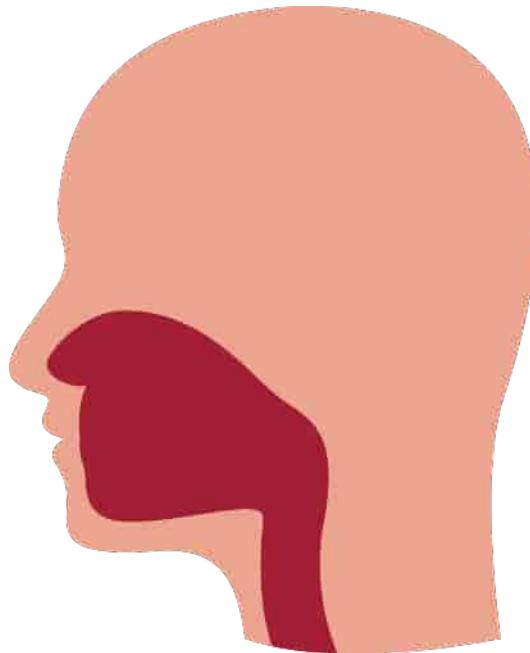
Note: for automated blood culture please do not put the sticker on the bar code.

CSF FOR CULTURE

General instructions

- The container should be sterile, screw-capped (available from general stores) labeled appropriately [see general instructions]. DO NOT COLLECT IN PENICILLIN BULBS SINCE THEIR STERILITY IS NOT MAINTAINED.
- Labeling – as in ‘blood’.
- Usually, 3 tubes of CSF are collected for biochemistry, microbiology, and cytology.
- If only one tube of fluid is available, it should be given to the microbiology laboratory.
- If more than one tube (1 ml each) is available, the second or third tube should go to the microbiology laboratory.
- Avoid exposure of CSF to excessive cold, heat or sunlight.
- IN CASE OF DELAY IN TRANSPORT TO LAB AFTER COLLECTION, STORE AT ROOM TEMPERATURE OR INCUBATOR ONLY. DO NOT REFRIGERATE.

COLLECTION OF UPPER RESPIRATORY TRACT SPECIMENS



Types of specimen

- Throat swab
- Nasopharyngeal swab
- Nasal swab

Requirement

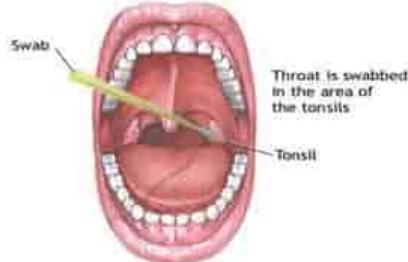
- Sterile swab
- Container - Sterile test tube, screw capped / cotton plugged to place the swab, VTM as in case of COVID 19 and flu
- Clean tongue depressor
- Source of light
- PPE in case of COVID 19 suspect

General instructions

- Follow airborne precautions and standard precautions
- In suspected cases of COVID 19, diphtheria, flu, swabs should be collected both from the throat and the nose
- In suspected cases of COVID 19 swabs should be collected both from the throat and the nasopharynx, as per ICMR guidelines
- In case of COVID 19, flu, use the special swab provided with the viral transport medium (VTM). Maintain cold chain in triple pack while transport.
- Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction

Procedure

- Perform hand hygiene.
- Wear appropriate mask / respirator / PPE for personal protection.
- Wear clean / sterile gloves.
- Ask patient to open his / her mouth without putting out his tongue and to say 'Ahhhhh....'



- While the patient is saying 'Ahhhhh', press down the outer two third of tongue with tongue depressor, using the left hand, enabling the tonsils and back of the throat to become visible.
- Introduce the swab with right hand between the tonsillar pillars and behind the uvula, while avoiding touching the tongue, cheeks, uvula, or lips.
- Rub the swab firmly against the inflamed part for 5 seconds while turning it round
- In case of suspected diphtheria, swab the membrane if present and If nothing abnormal is seen, swab the tonsils, the fauces and the back of the soft palate
- Take two swabs and immediately plug the same in sterile test tubes
- Specimens should be transported to the laboratory immediately after labelling and properly filling up the requisition form.

COLLECTION OF LOWER RESPIRATORY TRACT SPECIMENS

Types of Specimen

Lower Respiratory Tract Specimens include:

- Sputum -expectorated
- Sputum - induced
- Bronchial washings
- Broncho alveolar lavage [BAL]
- Mini-BAL
- Endotracheal aspirates

Sputum – expectorated

REQUIREMENT

- Lower Respiratory Tract Specimens include:
- Patients without complaints of cough with expectoration should preferably not be referred for sputum examination.
- For culture - The container should be sterile, wide-mouthed, screw-capped with a capacity of approximately 15-20 ml and labeled. The container can be procured from TRUSTlab Central stores. The procedure of collection should be explained to the patient. This includes:
 - Explaining the difference between saliva (spit) and sputum.
 - Explaining the cough etiquette and its importance.
- For sputum microscopy (acid fast bacilli) clean, screw capped containers are available at TRUSTlab Central stores.

**SPECIMEN COLLECTION,
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- Volume – 2–5 ml Number of specimens:
 - One for bacterial culture
 - Two (one early morning and one spot) for sputum AFB examination
- Collection should be done in a well-ventilated area away from people especially children.
- The patient should first rinse his/her mouth with plain water.
- The patient should open the container without contamination, breathe slowly and deeply, bend forward and generate a deep cough.
- Collect the expectorant in the container by pressing the rim of the container under the lower lip to catch the entire expectorated cough sample
- After collection, the cap of the container should be tightly screwed.
- Any spilled material on the outside should be wiped off with a tissue moistened with 1% sodium hypochlorite or alcohol, and care should be taken not to let any disinfectant enter the container.
- If the collection is done at home, visible contamination should be wiped off with household bleach.
- It should be ensured that the sputum sample is of good quality. A good quality sputum sample is thick, purulent and sufficient in amount (2–3ml).
- Fill the form and send sample immediately to laboratory.

**SPECIMEN COLLECTION,
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Sputum – Induced

- When sputum production is scanty, induction with physiotherapy, postural drainage, or nebulized saline may be effective.
- This procedure should be carried out in an area which is isolated and preferably under negative pressure or well ventilated without other humans around.
- Allow the patient to breathe 3% normal Saline for 10 minutes or until a strong cough reflex is generated.
- Collect the sputum thus generated (which tends to be watery) in a sterile screw capped labeled container (as for sputum above) and send to the laboratory immediately along with the duly filled requisition form.
- Mention that the specimen is induced sputum in order to avoid specimen rejection.

Endotracheal aspirate

- Indication – in intubated patients with suspicion of pulmonary infection
- Position the tip of the bronchoscope close to the segmental area corresponding to radiographic infiltrates.
- Instill 3 aliquots of 50 mL or 5 aliquots of 30 mL saline
- After the injection of each aliquot, gently aspirate through the suction channel.
- Send atleast 10 ml of the aspirate for microscopy and culture.
- Container – Sterile screw capped test tube

Bronchial aspirate

- These are collected by direct aspiration of material from the large airways of the respiratory tract by means of a flexible bronchoscope.
- Approximately 5 ml lavage is to be sent to the laboratory for microbiological examination /cytological examination.

Collection of Ear Swab

- Use sterile swab stick
- Collect under direct vision
- Do not instill antibiotic / antiseptic into the ear prior to collection
- Allow the swab to soak in the exudate for 10 seconds
- Place in sterile container (plugged / screw capped test tube), label and transport immediately.

Collection of Eye Swab (CORNEAL/ CONJUNCTIVAL)

- Moisten the swab in sterile normal saline
- Hold the swab parallel to the cornea and gently rub the lower conjunctiva
- Place in sterile container (plugged / screw capped test tube), label and transport immediately.

SKIN, NAIL AND HAIR FOR FUNGAL EXAMINATION

Collect skin scraping, hair and nail clippings in a Petri dish / test tube and maintain at room temperature.

a) Skin scrapings

- Identify the site of lesion from where collection is to be made. [An appropriate lesion is peripheral, erythematous, growing margins of typical ringworm lesion.]
- Inform the patient about the procedure.
- Collect specimens with strict aseptic precautions.
- Make the patient sit comfortably.
- Clean the identified lesion thoroughly with 70% alcohol to remove the surface bacterial contamination.
- Using a sterile scalpel blade surface collect multiple scrapings from the identified lesion preferably from the edge of the lesion including the adjacent healthy skin.
- Collect the specimen in a petri dish, filter paper or clean paper.

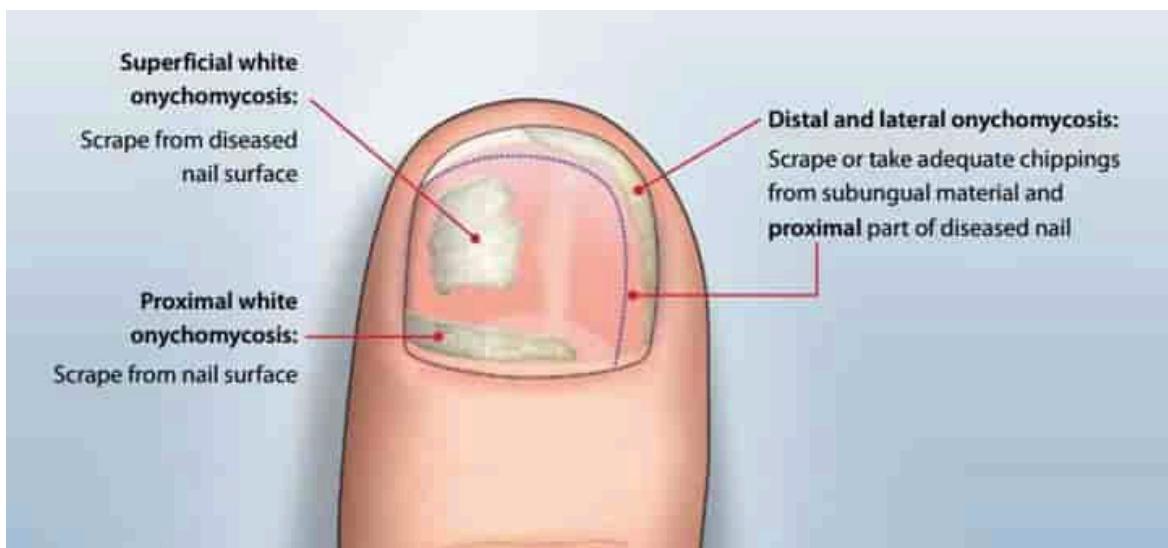
b) Nail

- Clean the affected nail with spirit
- Collect debris under the nail with a scalpel in Petri dish
- Pick up flakes after wetting loop with sterile saline from Petri dish for processing
- If the nail is avulsed then it should be cut into small pieces for processing.

c) Hair

- Hair should be collected from areas of scaling or alopecia.
- Clean the affected area with spirit.
- With sterilized forceps, pluck hair or stubs (at least 10-12) in grey patch or scrape with scalpel in black dot type of hair infection.

SPECIMEN COLLECTION, TRANSPORTATION & HANDLING MANUAL



d) Skin Biopsy

- Decontaminate skin with 70% methylated spirit / alcohol.
- Select the edge of the lesion.
- Take a biopsy with an autoclaved instrument under all aseptic measures.
- Cut biopsy tissue into small pieces and crush in a mortal and pestle.

e) Mycetoma granules

- From suspected mycetoma, look for granules in the lesions using hand lens.
- Wash the granules in several changes of sterile distilled water
- Crush the granules and then inoculate
- If granules are absent collect the purulent/necrotic material.

COLLECTION OF CONJUNCTIVAL DISCHARGE

Types of specimen

Discharge from the eye(s).

Inappropriate specimen transport device; mislabeled specimen; unlabeled specimen; specimen received after prolonged delay (usually more than two hours); specimen received in expired transport media.

Specimen collection

- Pull down the lower eyelid so that the lower conjunctival fornix is exposed.
- Swab the fornix without touching the rim of the eyelid with the sterile cotton swab.
- Place the swab immediately in a bacterial transport medium or, if the specimen is brought to the laboratory immediately, in a sterile test tube with 0.5 mL of buffered saline (pH 7).



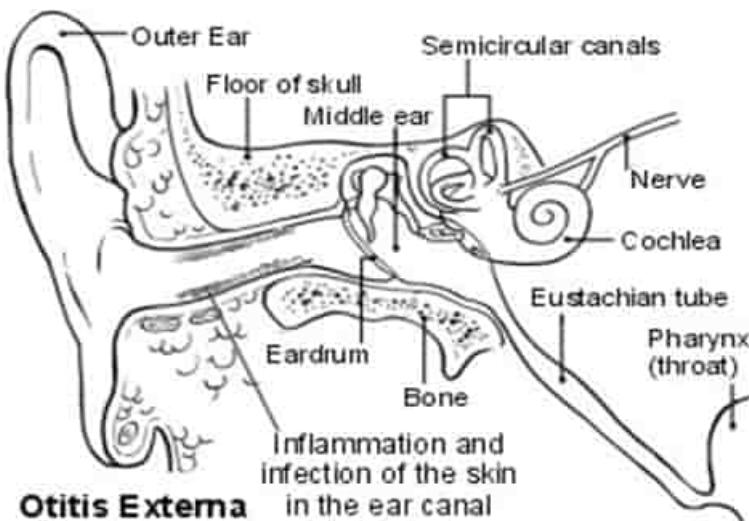
- Ensure to collect sufficient amount on the swab.
- Eye specimen should be processed immediately because tears contains lysosomes which may kill the organism.
- The collected sample should be stored in refrigerated condition (2-8 0C).
- All bacteria isolated in fair amounts and not resembling contaminants will be identified and tested for antibiotic susceptibility, including susceptibility to chloramphenicol.

COLLECTION OF EAR DISCHARGE

Pus from the external or middle ear.

Criteria of specimen rejection

Inappropriate specimen transport device; mislabeled specimen; unlabeled specimen; specimen received after prolonged delay (usually more than two hours); specimen received in expired transport media.

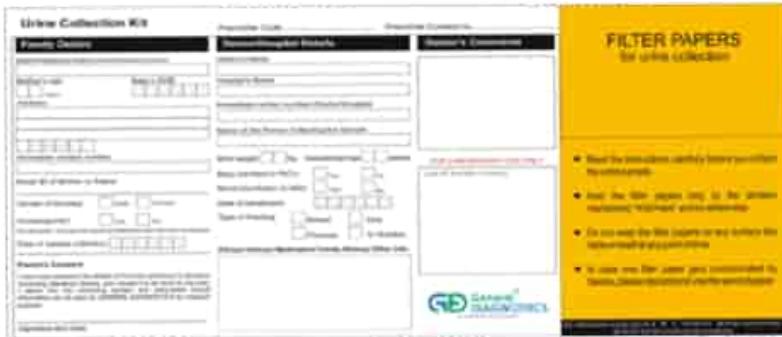


Specimen collection

- Collect a specimen of the discharge on a thin, sterile cotton wool or Dacron swab.
- Place the swab in a container with the transport medium, breaking off the swab stick to allow the stopper to be replaced tightly.
- Label the specimen and send it to the laboratory.
- Sample should reach the lab and get processed within 2 hours after collection.
- The collected sample should be stored in refrigerated condition (2-8 °C).

SAMPLE COLLECTION FOR NEWBORN SCREENING

Urine on Filter paper- UFP



- Make sure that all the information in the Card is duly filled and recheck with the Parents.
- The Sample can be collected with one of the methods mentioned Method

Method - 1:

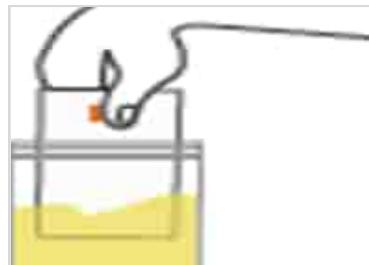
- a) Hold the green portion of the filter paper to collect the sample



- b) Hold the filter paper close to the genital area of your baby until your baby passes urine directly on it.

Method - 2 :

- a) Hold the green portion of the filter paper to collect the sample



- b) Collect 20-25 ml of urine in a clean sterile plastic container. Hold the filter paper from the colored end and soak it in the collected urine sample.
- c) Thoroughly Air Dry by hanging with clip or keeping on a clean, flat and STERILE surface for around half an hour to one hour.
- d) Mail completed form with sample to the laboratory soon after collection and drying the same.



Dried blood spot- DBS

Make sure that all the information in the Card is duly filled and recheck with the Parents.

- Visually demarcate the site



**SPECIMEN COLLECTION,
TRANSPORTATION & HANDLING MANUAL**

- The shaded area (//////////////) indicates safe areas for puncture site.
- The safest area can be visualized as median to a line drawn posteriorly from the middle of greater toe to the heel or lateral to a line drawn from between the 4th and 5th toe to the heel.
- Warm the prick site by massaging gently or with soft cloth, moistened with warm water, for three to five minutes



- Cleanse site with alcohol prep & Wipe DRY with sterile gauze pad
- With the lancet, puncture the heel skin with one continuous, deliberate motion at a slight angle (a little less than 90 degrees).



- Wipe away first blood drop with sterile gauze pad (as it is likely to contain tissue fluids that can contaminate the specimen)
- Allow another LARGE blood drop to form Lightly touch filter paper to LARGE blood drop.



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- Thoroughly dry blood spots on a sterile, dry, clean, flat, nonabsorbent surface for around 30 mins.



- Mail the completed form with the sample to the laboratory soon after collection and drying the same

CYTOPATHOLOGY

The success of a laboratory's cytopathology program depends on the combined efforts of the referring doctor and his/her staff and the laboratory, obtaining the patient's medical history and an adequate, properly fixed specimen. A final report, using descriptive cytopathology terminology and an accurate interpretation are required to assure appropriate follow up.

a) Cytopathology test requisition form

In addition to the general information on the test requisition form we strongly recommend providing the patient source of material submitted (cervical, endocervical, vaginal, or other gynaecologic or non-gynaecologic site).

For the Gynaecologic sample, following information is must:

- Last Menstrual Period (LMP)
- Pertinent clinical information (routine examination, pregnant, postpartum, hormone therapy, oral contraceptives, hysterectomy post-menopausal, pelvic radiation, per speculum and per vaginal examination findings etc.).

For Non-Gynae Specimens:

- Source and specific site (left, right, quadrant, etc.)
- Nature of lesion (solid/cystic, mobile/fixed, functional/non-functional etc.)
- Any other significant history (previous survey, presence of other masses, previous abnormal findings)
 - Mammographic, X-ray or other imaging findings.
 - Nature of aspirate.

Collection for Non-Gynae specimens

Solid organ cytology collection

Appropriate Fixatives for Non-Gynaecological Cytology specimens include

- Ethyl alcohol/Isopropyl alcohol/Commercial Cytospray
- Mixture of 50% ethanol + 50% ethanol + 50% ether

Specimen collection:

- Preferably label the slide with patient's name.
- From the total number of slides prepared from the aspirate, fix one containing adequate material, immediately with any of the above mentioned fixatives for 3-5 minutes. Allow fixative to dry thoroughly before packaging slides for transport.
- Allow the remaining slides to air dry.
- Pack the fixed smear slide in either a paper wrapping (3-4 layers thick) or an appropriate slide mailer to avoid slide breakage. Mention that the smear is fixed either on the slide, wrapped paper or container.
- Also, pack the remaining air-dried slides similarly as described above in step
- Place all these slides together, label them properly with patient details and transport them to the laboratory taking care that the slides do not break in the transport.

Fluid collection for cytology

- Complete test requisition.
- All fluids need to be transported immediately to the laboratory. If transport of the fluids will be delayed more than 24 hours, add an equal volume of 50% ethyl alcohol or mixture of 50% ethanol + 50% ether to the fluid (if sample size is too large to accommodate this volume, a well-mixed aliquot of the fluid with an equal volume of fixative may be utilized). If transport time will be less than 24 hours, and if fixative is not available, the fluid should be refrigerated or kept on wet ice until transport to the laboratory.
- Place fluid/fixative mixture in a tightly capped, leak proof, labeled container (label the container wall, not the lid).
- Syringes are not acceptable as specimen containers.

The following table lists out some of the frequent cytology samples with the submission requirement:

Cytology samples –Source

Breast Cyst Aspiration:	If aspirate is scanty, fluid may be smeared one drop at a time, on clean and dry slides and immediately fixed. If aspirate is abundant, mix material with an equal volume of fixative.
Breast Secretion (Nipple discharge):	Drops of fluid from the nipple are smeared directly on clean glass slides and fixed immediately with Cyto spray or immersed in alcohol for 3-5 minutes.
Bronchial brushing:	Swirl brush/brushes used to prepare bronchial brushing slides in a container of fixative to dislodge additional specimen.
Effusions:	Mix material with an equal volume of fixative
Endometrial washings:	Mix material with an equal volume of fixative
Esophageal Brushing:	Swirl brush/brushes used to prepare slides in a container of fixative to dislodge additional specimen. Submit liquid specimen together with test requisition.
Esophageal Washings:	Mix material with an equal volume of fixative
Gastric Washings:	Mix material with an equal volume of fixative

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Fine Needle Aspiration:	Fix/ and air dry the labeled smears with the fixative and transport. Air dried slides should be labeled as such.
Gastric brushing:	Swirl the brushes in a container of fixative. The container wall should be labeled with the patient's name. Submit liquid specimen together with test requisition.
Lymph Node (touch prep):	Fix immediately in alcohol or use Cytospray. Air dried slides should be labeled as such.
Peritoneal, Pericardial & Pleural fluid:	Mix material with an equal volume of fixative
Sputum:	Submit early morning deep cough specimen prior to any food ingestion. Have patient rinse mouth with plain water before sputum is collected. Collect separate specimens on 3 consecutive mornings. Do not pool specimens. Mix material with equal volume of fixative.
Urine:	Submit all specimens in an equal volume fixative

Collection of Cervical & Vaginal Smears

- Most of the Gynaecological smears are collected by Referring Physicians in a coplin jar containing 95 % alcohol fixative, labeled. and sent to the laboratory reception counter, where they are. verified and sent to Cytology section of the lab.
- Some patients are directly referred to the lab to collect Pap. smear. A trained technician takes the smear.

The procedure is as follows:

The patient is comfortably placed on an examination table, a Sim's speculum is inserted in to the vagina and the cervix is exposed. A wooden spatula is inserted into the cervix, after cleaning the cervix with a sterile cotton swab. A pan cervical smear is taken from squamous columnar JN/Transition zone by rotating spatula clock wise direction for 360°. The spatula is immediately removed from the vagina and the contents are placed on a clean labeled and thin slide is made in a wet condition, at once it is placed in the fixative and sent to Cytology lab. Vaginal smears are taken by scraping the walls of the vagina with the spatula and thin smears are made on a clean labeled slide and placed in fixative solution and sent to cytology lab.

Other body fluids: (C.S.F., Peritoneal, Pleural, Bronchial aspiration, stomach Wash, Urine etc. (Clear fluids):

- Take 5 cc of the fluid, and concentrate the fluid by centrifuging at 2500/RPM for 15 minutes.
- Decant the supernatant and re-suspend the sediment in a drop of the respective fluid.
- Put a drop of this concentrated fluid on a clean glass slide, add one drop of pooled plasma or Mayer's albumin and make thin smears and immediately transfer one to the fixative bottle containing 95 % alcohol.
- Close the lid keep it in the fixative for 1 hour.
- The other slides can be air dried
- Send the unstained slides in slide mailers to prevent breakage along with the re-suspended fluid.

Turbid and Cloudy fluids:

- The fluids usually contain inflammatory exudates and protein,
- They are concentrated by taking 5 ml, of the fluid and add 5 drops of 2 % acetic acid, which will lyse RBCs and remove necrotic debris
- Centrifuge at 2500 /RPM for 15 minutes, discard the supernatant
- Re-suspend the sediment in 2 drops of the fluid with the help of a pasture pipette,
- Place a drop each on two clean labeled slides, then place in fixative containing of 95% alcohol close the lid,
- Allow them to stay in fixative for 1 hour.
- Some of the slides can be air dried

Sputum:

- Sputum is collected in a clean dry receptacle, preferably overnight by coughing.
- Avoid saliva as far as possible.
- Close the lid and send it to the laboratory.
- The identification is put on the collection jar.
- Handle with care taking all aseptic precautions in the laboratory, preferably under a bio safety hood
- With the help of a wire loop pick sputum particles including shreds of mucous
- Place it on a clean labeled glass slide and spread the slide for about 3 x 2 cm area and place it immediately in a fixative bottle containing 95 % alcohol and recap. Some smears can be air dried
- After one hour the smears are ready for staining.

FNAC:(Fine Needle Aspiration Cytology)

FNAC aspiration is performed and smears are prepared by only qualified and experienced pathologists/radiologists and smears are sent to the laboratory. The slides are labeled, and some are immediately placed in a 95% alcohol container for fixation. Some air-dried smears are kept for staining by Romanowsky stains MGG/Giemsa/Leishman's).

HISTOPATHOLOGY

Specimen Handling-Routine histopathology

- Immediately place each specimen in a tightly secured container with 10% neutral buffered formalin. Specimen must be totally immersed in formalin. Do not allow specimen to dry. The volume of the formalin should be preferably ten times the volume of the specimen.
- Use a separate container for each separately identified specimen.
- Do not crush the specimen with forceps, hemostats, or other instruments. Cautery will cause heat artefact. Do not freeze formalin fixed specimens.
- Do not force a large specimen into a small container. Formalin must surround the specimen for fixation.
- Label each container wall (not the lid) with patient's name and source of specimen. Place one of the peel off labels onto each specimen container.
- Complete a histopathology test requisition and send with specimen(s). Only one histopathology test requisition is needed per patient. Each container and specimen must be separately identified on the test requisition. Also mention the total number of specimen when >1 containers are sent for the same patient on the "Histopathology Test Requisition Form".
- The test requisition should contain pertinent clinical information including patient's name, age, gender, clinical information, anatomic location of tissue removed and radiological findings for bone and brain cases.
- If there is any delay in transportation large specimens should be cut into two halves packed with formalin soaked cotton and placed in the fixative so that there is no autolysis in the deeper parts of the Specimen.

Type of examination and submission requirements

- Routine Histopathology: as stated above
- Immunohistochemistry (IHC)
- Formalin fixed paraffin embedded blocks of representative tissues are to be submitted. Preferably submit one routinely stained H&E section of the block with appropriate clinical history and copy of histopathology report.
- Alternatively, submit specimens as for Routine Histopathology.

- Special Stains: Submit specimens as for Routine Histopathology or submit formalin fixed paraffin blocks of representative tissue. Attach Histopathology report, relevant clinical history and one histology section of the block submitted.

PATIENT HISTORY IS A MUST FOR A CORRECT AND TIMELY REPORT. IN CASE OF LACK OF CLINICAL HISTORY, THE REPORT IS NOT RELEASED AND KEPT IN "PENDING" STATUS. THE PERIOD FOR "PENDING" STATUS IS 15 DAYS FOR HISTOPATHOLOGY.

If the history is not provided in the stipulated period the report will be released with appropriate comments.

Sample Rejection Criteria (Histopathology and Cytology)

Listed below are the reasons for specimen rejection/delay in release of reports:

Major Criteria:

- Fixative leaked from container rendering tissue unfixed/autolysed and foul smelling.
- Slides/Paraffin Blocks broken beyond repair on receipt.
- No Patient details mentioned on TRF.
- Mismatch between details on the specimen label and TRF.
- Action to be undertaken:
 - Inform the concerned pathologist as early as possible.
 - Immediate communication to the respective PSC/customer via telephone/email.

Minor Criteria:

- No proper patient identification on specimen container or slides.
- No clinical details mentioned on TRF.
- Slides and TRF received separately and posing difficulty in proper identification.
- All attempts are made not to REJECT samples.
- Samples will be processed and stained, but no report will be issued.
- The TAT for such samples is from the time the details/clarifications are obtained.

The following **ARE ACCEPTABLE** specimens for pathological evaluation:

- Specimens directly from Operation Theater, previously procured, fixed in 10% formalin.
- Specimen directly from Operation Theater, previously procured, fresh. Smaller fresh specimens should be kept moist in saline or a transport media such as RPMI.
- OPD biopsies fixed in 10% formalin.
- Autopsy specimens, labeled with the autopsy number, in 10% formalin with summary of autopsy report.
- Any foreign bodies such as ports, stents, etc. for which a Surgical Pathology order has been received. In general, these will be handled as "gross only" cases for which no samples are submitted for microscopic evaluation. These can be submitted in dry specimen containers. If there is adherent tissue, that material may be submitted for paraffin processing at the discretion of the resident and attending pathologist. These should be submitted in formalin to prevent decay, even if the tissue will not be processed for examination.
- Usually tooth is examined only grossly however adherent tissue submitted is only processed for microscopic examination.
- Consult materials consisting of fixed wet tissue, paraffin blocks, and/or glass slides.
- Frozen material if requested by an attending Consultant pathologist only.
- Frozen Section specimens, consultation completed, in 10% formalin.

SPECIMEN PRESERVATION

A) Specimens in 10% neutral buffered formalin:

Anything received in 10% neutral buffered formalin can be held at room temperature until grossed by the resident. Refrigeration (2-8°C) is not needed and should be avoided so as not to slow down the chemical reaction. These specimens may be received in pre-filled formalin containers, which the Histopathology laboratory provides, or in generic specimen cups to which 10% formalin has been added. 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, contact the resident to add more formalin or transfer to a larger container. The volume of fixative should be at least two times that of the specimen.

B) Specimens received fresh:

Anything received fresh (without a fixative such as formalin or alcohol) must be brought to the attention of the resident to ensure proper handling. In the interim, fresh specimens must be refrigerated (0-8°C) until the resident makes sure the sample has been procured. Large samples from the Operation Rooms must be covered with Parafilm or similar material. Smaller samples should be kept moist with a Tyvec or similar pad moistened with saline or RPMI and submitted in a sealed specimen container.

COAGULATION

Collection of blood for coagulation testing through intravenous lines that have been previously flushed with heparin should be avoided, if possible. If the blood must be drawn through an indwelling catheter, possible heparin contamination and specimen dilution should be considered. When obtaining specimens from indwelling lines that may contain heparin, the line should be flushed with 5 mL of saline and the first 5 mL of blood or 6-times the line volume (dead space volume of the catheter) be drawn off and discarded before the coagulation tube is filled. For those samples collected from a normal saline lock (capped off venous port) twice the dead space volume of the catheter and extension set should be discarded.

After blood collection, there is progressive degradation of the labile coagulation factors V and VIII, leading to increasing prolongation of the APTT and PT. The allowable time interval between specimen collection and sample testing depends on the temperature encountered during transport and storage of the specimen. Allowable time intervals are as follows:

PT specimens, uncentrifuged or centrifuged with plasma remaining in the capped tube above the packed cells, or as centrifuged plasma separated from the cells, should be kept at 18 to 24°C and tested no longer than 24 hours from the time of specimen collection. PT specimens should not be refrigerated (during storage).

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aPTT specimens that are uncentrifuged with plasma remaining in the capped tube above the packed cells should be kept at 18 to 24°C and tested no longer than 4 hours after the time of specimen collection. aPTT specimens that are centrifuged and plasma separated from cells can be kept for 4 hours at 2 to 8°C or 18 to 24°C.

Samples for unfractionated heparin testing should be centrifuged within one hour from the time of specimen collection. Samples for other coagulation factors (e.g. thrombin time, protein C, factor V, factor VIII) have variable stability and should be kept in the same manner as aPTT samples. PT or aPTT testing cannot be performed within these times, platelet-poor plasma should be removed from the cells and frozen at -20°C for up to 2 weeks or at -70°C for up to 12 months. If a laboratory has established an allowable time interval different than that detailed above, data must be available to verify that coagulation testing is valid in the time interval established.

1. Specimen is to be collected in 3.2 % sodium citrate tube (light blue top vacutainer). All other anticoagulants (heparin, EDTA, oxalate or NaF) are NOT acceptable.

- A fasting sample is preferred.
- Specimen must be sent with a duly filled requisition form(mandatory).
- Tourniquet should not be applied for more than one minute.
- To avoid contamination of the sample with tissue thromboplastin or heparin, follow the guidelines below since these substances may alter results.
- The venipuncture must be clean with minimal trauma.

2. Sodium citrate tube has to be the 2nd tube in the order of sample draw, if drawing a blue top tube for coagulation tests,(other than PT and PTT),a red tube(2-3 cc) must be drawn first to avoid contamination from tissue thromboplastin, which can yield false coagulation results . Blue tubes must also be completely filled to avoid erroneous test results.

[Note: When using a winged blood collection set for venipuncture and a coagulation tube is the first tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection tubing dead space and to assure maintenance of the proper anticoagulant/Blood ratio and need not be completely filled. The discard tube should be a plain red(no additive)or coagulation tube.]

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3. If sample is drawn from an indwelling catheter, flush with 5mL of saline and discard the first 5mL of blood collected before collecting the specimen for coagulation testing. Blood should not be collected from heparinised lines.

4. Hemolysed samples are not acceptable because Erythrocytin (similar to thromboplastin) released from RBCs may affect test result.

- The tube is gently inverted 8-10 times after blood collection to facilitate proper mixing of blood and anticoagulant.
- Check for adequate dilution. Vacutainer must be filled up to the indicated mark to ensure the proper 9:1 blood to anticoagulant ratio.
- Make sure the samples are not clotted. Clotted specimens are repeated.
- Samples in vacutainers collected at local centers/ hospitals (which do not prepare platelet poor plasma on site) and main lab should reach the department within 1-1.5 hours after collection, so that they can be tested within 2 hours at room temperature. These should not be refrigerated or frozen, since cold activates Factor VII.
- Specimens collected at outstation/ local collection centers / satellite labs where these tests are not performed, platelet poor plasma (PPP) prepared within 1 hour; immediately frozen and transported frozen is the only acceptable choice. At last two 1.0-1.5 mL aliquots of PPP per test are required for analysis. For each additional test add one aliquot.
- Centrifuge the sample at **3000 rpm for 10 minutes** within 1 hour of collection, to separate the plasma.
- All samples with hematocrit >55% are repeated. If the plasma in the tube after centrifugation appears to be <50% of the total volume, the hematocrit is determined. Do not prepare PPP in these cases and ask the patient to contact the Hematology Department in National Reference Lab.

PARASITOLOGY

a) Blood parasites: Plasmodium (Malaria), Microfilaria (Filarial worms), Babesia, and Trypanosoma (Trypanosomiasis)

- Submit one lavender top (EDTA) and minimum of one thick and one thin blood film taken from peripheral blood during the febrile episode.
- Ideally, for thick and thin blood films collect peripheral blood via finger prick. Blood collected by vein-puncture and preserved with EDTA is also acceptable.
- To prepare a thick smear from a finger prick, blood specimens must be collected from the tip of the “ring” finger on the palmar surface.
- Warm the skin area to be punctured with sterile, warm saline.
- Clean and disinfect skin with 70% alcohol.
- Wipe dry or air dry. Be sure the finger is thoroughly dry prior to pricking.
- Stick the finger with a sterile disposable lancet, deeply enough to collect a sufficient amount of free flowing blood for film preparation. Do not squeeze finger to remove the blood.
- Holding a clean glass slide, touch the underside to the puddle of blood which has collected at the puncture site. Right the slide and allow the thick film to dry.
- Repeat the procedure and “feather” the blood drop to prepare the thin film.
- After collection, apply pressure to the puncture site until bleeding stops.
- It is recommended that the blood be drawn when the patient presents with symptoms at 6- hour intervals for 36 hours.
- Obtain patient history as an aid in diagnosis. This should include visits to any endemic area and the date of return.
- Print the patient's name/identification on one end of the slides.
- If Wuchereria bancrofti or Brugia malayi are suspected, draw blood between 10 pm and 4 am.
- If diurnal Loa is suspected, draw blood between 10 am and 2 pm. A hetaran provocative test may be carried out if recommended by a doctor to enable collection of blood for microfilaria during day time.
- Specimens collected after the initiation of drug therapy hamper parasite identification.
- Transport to the laboratory at room temperature as soon as possible and no more than 24- 48 hours after collection avoiding extremes of heat and cold.

b) Duodenal/ Gastric Aspirates:

- Collect the duodenal/gastric aspirates and the fluid in both 10% formalin and a sterile transport vial within 30 minutes of collection.
- Indicate on the specimen vial the source of the specimen.

c) Eye: Acanthamoeba Culture:

- Submit contact lens fluid in a sterile plastic tube.
- For conjunctivitis: Use a pre-moistened sterile culture swab by rolling it over the conjunctiva behind the eyelids. Immediately replace the swab back into the culture transport media and send to the laboratory.
- For corneal infection: Collect corneal scrapings by instilling one or two drops of topical anesthetic. Using a sterile corneal spatula gently scrape corneal ulcers or lesions. Place them in sterile saline solution and submit within 24-48 hours refrigerated. Do not freeze.

d) Parasite identification (Worm, Tick, Larva, Other Insects):

- Submit the entire organism in 70% Isopropyl alcohol in a clean screw-cap container.
- Transport at room or refrigerated temperature (stable 2 months).

e) Skin

- Microfilaria (filarial worms)
- Epidermal skin clips is the specimen of choice if onchocerca volvulus or Mansonella streptocerca infections are suspected.
- Transport at room temperature as soon as possible and no more than 24-48 hours after collection avoiding extremes of heat and cold.

f) Sputum and lower respiratory tract specimens *Pneumocystis carinii*.

- The patient should rinse mouth with water before sputum is collected.
- The induced sputum specimens from an early morning sample obtained after a deep cough is acceptable.
- Do not pool multiple samples collected during 24-hours period.
- Instruct the patient to avoid adding saliva or nasopharyngeal discharges to the sputum sample to avoid contamination with indigenous microorganisms.
- Collect the sputum in a sterile screw-capped container. Twist the cap securely closed after placing the specimen in the container.
- Collect lower respiratory tract specimens by bronchoscopy or transtracheal aspiration using diligence to avoid contamination and leakage during transport.
- Transport refrigerated within 24-48 hours.

MOLECULAR/ VIROLOGY

This section deals only with few of the viruses, which require special means of sample collection from different sources.

a) Ophthalmic specimen:

Collection: Apply local anesthetic to the eye, expose upper and lower conjunctiva. Using a Dacron or cotton tipped swab, vigorously wipe both upper and lower conjunctiva surfaces to ensure that the entire conjunctiva surface is sampled.

Slide Preparation: Roll the specimen swab using slight pressure on a Teflon coated/albumenized glass microscope slide. Allow the specimen to air dry at room temperature (15–30°C). Fix in fresh acetone at room temperature for 10 min. Store at 4°C overnight if the specimen is not being stained immediately.

b) Herpes simplex virus:

Specimen must be collected so as to contain as much infected material as possible. Cotton tipped swabs with metal, plastic or compressed paper stems are recommended. Wooden stemmed swabs should not be used. The specimen swabs have to be transported in special transport media kit available. Use 1 ml of the specimen transport medium for each swab.

c) Skin ulcer/blister/vesicle/pustule:

Ulcers should be firmly rubbed with the swab in order to pick up the infected cells and exudates from the base of the ulcer. Vesicles should be carefully opened, fluid is collected on the swab and base of the lesion rubbed with the swab. Pustular lesions should be treated as for vesicles.

Any crusts should also be collected. Place the swab in 1 ml transport medium in a glass vial. Transport immediately refrigerated or store at 2–8°C for no longer than 3 days.

d) Cervical swabs:

Firmly swab any visible cervical lesion or the cervix with cotton tipped swabs. Withdraw without touching the vaginal surface and place in 1 ml transport medium in a glass vial. Transport immediately refrigerated.

If there is delay, specimens may be stored at 12–8°C for no longer than 3 days.

Note: Use of creams, ointments, lotions, ice, alcohol, Betadine solutions, Zinc reduces viral yield significantly. Use of such remedies should be avoided prior to sample collection.

e) Saliva collection:

The salivary collection is preferred for certain tests due to the following reasons:

Ease of collection – Minimal training is necessary and individuals can collect their own sample.

Non-invasive method – It is much easier to recruit subjects for studies that utilize saliva. Studies looking at stress responses with cortisol are not affected by the stress of having a venous blood sample taken.

Cost effective – Supplies for collecting samples are much less expensive.

Safety – Saliva is classified as a Category B substance and is subject to the relevant legislation, it is not as hazardous as blood, is safer to collect, handle and ship.

Accuracy – Saliva levels are highly correlated with serum levels.

Saliva collection supplies & devices usually comprise of the following: Oral Swab, Swab storage tube, conical tube, etc. Commercially available saliva sampling devices may also be used. With many POCT kits the salivary collection system forms an integral part; in such cases the kit prescribed procedure is followed for collection.

f) Nasopharyngeal Swab:

Insert dry flexible synthetic swab into nostril and back to nasopharynx. Leave in place for a few seconds. Slowly remove swab while slightly rotating it. Use a different swab for the other nostril. Put tip of swab into vial containing VTM, breaking applicator's stick. Nasal Swab is collected from the anterior turbinate. Label the container containing the culture specimen clearly, indicating the ID number of the patient, the nature of specimen and site from which it was collected.

g) Oropharyngeal / Throat Swab:

- Instruct the patient to tilt his / her head back and breathe deeply. Gently depress the tongue with a sterile tongue blade to visualize the tonsillar fossa and posterior pharynx.
- Instruct the patient to phonate a long 'Ah' to lift the uvula. Firmly swab between tonsillar pillars posterior uvula and posterior pharynx taking care not to touch the lateral walls of the buccal cavity. Take minimum two swabs each for Infectious procedures.
- Any purulent exudates should also be sampled.
- After collection, place the swab immediately into a sterile tube.
- Label the container containing the culture specimen clearly, indicating the ID number of the patient, the nature of specimen and site from which it was collected.

COVID 19 RNA RT PCR ASSAY

SAMPLE REQUIRED - Nasopharyngeal swab / oropharyngeal swab in VIRAL TRANSPORT MEDIA.

MATERIALS REQUIRED

- N95 Masks
- PPE kits
- Nitrite gloves
- Alcohol based hand sanitizer (70%)
- Alcohol swabs
- 1% hypochlorite solution
- Sample vials and VTM(viral transport media),
- Absorbent material (cotton, tissue paper)
- Paraffin
- Cello tape
- Scissor
- Leak proof container (ziplock pouch, cryobox, 50 ml centrifuge tube and plastic container).
- Thermacolbox/Ice boxes/Vaccine carrier, Cellotape, Biohazard yellow bags, Hard frozen Gel Packs and Marking pens.

Before collection guidelines

All Universal Biosafety Precautions should be followed throughout the procedure of sample collection.

Wear PPE (Personal Protective Equipment) like Apron, Hand gloves, N95 Mask, goggles, face shield and boots /shoe cover) as per the donning & doffing procedures given below.

Ask the person to sit comfortably and also ask his/her name and age. Explain procedure to the patient.

DONNING

Preparation of donning

- Procedure must be carried out exclusively in clean room identified for donning procedure. No exposed skin or hair of the laboratory personnel should be visible at the conclusion of the donning process.
- The laboratory personnel must have adequate liquid or drinks in order to avoid interruption of sample collection due to thirst or dehydration. PPE requires a tight fitting therefore; one may lose fluid due to perspiration and as a result dehydration may occur.
- Remove Personal Clothing and Items and Change into surgical scrubs (or disposable garments). No personal items (e.g., jewellery, watches, cell phones, pagers, pens) should be brought into patient room.
- Visually inspect the PPE to be worn to ensure it is in serviceable condition, all required PPE and supplies are available, and that the sizes selected are correct for the laboratory personnel.

Donning procedure

- Perform hand hygiene with hand sanitizer and allow hands to dry before moving to next step.
- Wear on first pair of inner gloves.
- Put on Apron/gown. Ensure it is large enough to allow unrestricted freedom of movement. Ensure cuffs of inner gloves are tucked under the sleeve of the gown or coverall to prevent skin from getting exposed.
- Put on Shoe Covers.
- Put on N95 mask. Complete a user seal check.
- Put on full-body disposable apron to provide additional protection to the front of the body against exposure to body fluids or excrement from the patient.
- Put on second pair of gloves (with extended cuffs). Ensure the cuffs are pulled over the sleeves of the gown or coverall.
- Put-on full-face shield over the N95 mask to provide additional protection to the front and sides of the face, including skin and eyes.
- The laboratory personnel should be comfortable and able to extend the arms bend at the waist and go through a range of motions to ensure there is enough range of movement while all areas of the body remain covered.

- Disinfect outer-gloved hands with alcohol rub and allow drying prior to sample collection or sample processing.

DOFFING

Preparing for Doffing

The doffing process is sequence of the procedure that the PPE has been removed properly. Prior to doffing PPE, must remind laboratory personnel to avoid reflexive actions that may put them at risk, such as touching their face.

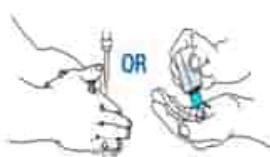
Doffing procedure

- Inspect the PPE to assess for visible contamination, cuts or tears before starting to remove. If any PPE is visibly contaminated, then disinfect with 70% ethanol.
- Disinfect and Remove Outer Gloves without contaminating the inner gloves
- Inspect inner gloves for any visible contamination (Note: Change Inner Gloves in case if there are visible tears and wears. Remove and discard gloves taking care not to contaminate bare hands during removal process and don a new pair of gloves).
- Remove the apron away from the body taking care not to contaminate the Apron/Gown. Roll the apron inside out and discard safely in the bio-hazard box.
- Disinfect the inner gloves with 70% ethanol and inspect PPE for any visible contamination.
- Remove the full-face shield by tilting the head slightly forward, grabbing the rear strap and pulling it over the head, gently allowing the face shield to fall forward and discard. Avoid touching the front surface of the face shield.
- Disinfect the inner gloves with 70% ethanol and remove apron/gown.
- Laboratory personnel can seek assistance by the trained observer to remove the apron/gown. Avoid contact of scrubs or disposable garments with outer surface of apron/gown during removal. Pull apron/gown away from body, rolling inside out and touching only the inside of the gown.
- To remove apron/gown, with one hand unzip the suit from inside. Unzip or unfasten completely before rolling down and turning inside out. Avoid contact of scrubs with outer surface of coverall during removal, touching only the inside of the apron/gown.
- Disinfect the inner gloves with 70% ethanol and remove the N95 respirator by tilting the head slightly forward, grasping first the bottom tie or elastic strap, then the top tie or elastic strap, and remove without touching the front of the N95 mask. Discard N95 Respirator.

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- Disinfect the shoe covers with 70% ethanol and remove while sitting down on comfortable chair.
- Disinfect the inner gloves with 70% ethanol Remove inner gloves safely and clean your hand with hand sanitizer.
- Perform hand hygiene and wear clean slippers or shoe.
- Remove surgical scrubs.

Process Flow charts

Donning Procedure	Doffing Procedure
Perform hand hygiene 	Disinfect the Outer gloves 
Wear on first pair of inner gloves 	Disinfect and Remove Outer Gloves 
Put on Apron/gown 	Remove the apron 
Ensure cuffs of inner gloves are tucked under the sleeve of the gown 	Remove the full-face shield 

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Put on Shoe Covers 	Remove the N95 respirator 
Put on N95 mask 	Remove Shoe Covers 
Put-on full-face shield over the N95 mask and surgical hood 	Remove inner gloves 
Disinfect outer-gloved hands with 70% ethanol 	Perform hand hygiene 

COLLECTION PROCEDURE

Nasopharyngeal swab (NP SWAB)/Oropharyngeal (Throat) swab (OP SWAB)

NP swab:

Insert minitip swab with a flexible shaft (wire or plastic) through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.



OP swab:

Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.



SAMPLE PACKAGING AND TRANSPORTATION

After collection of NP or OP place it immediately in VTM (Viral transport media)

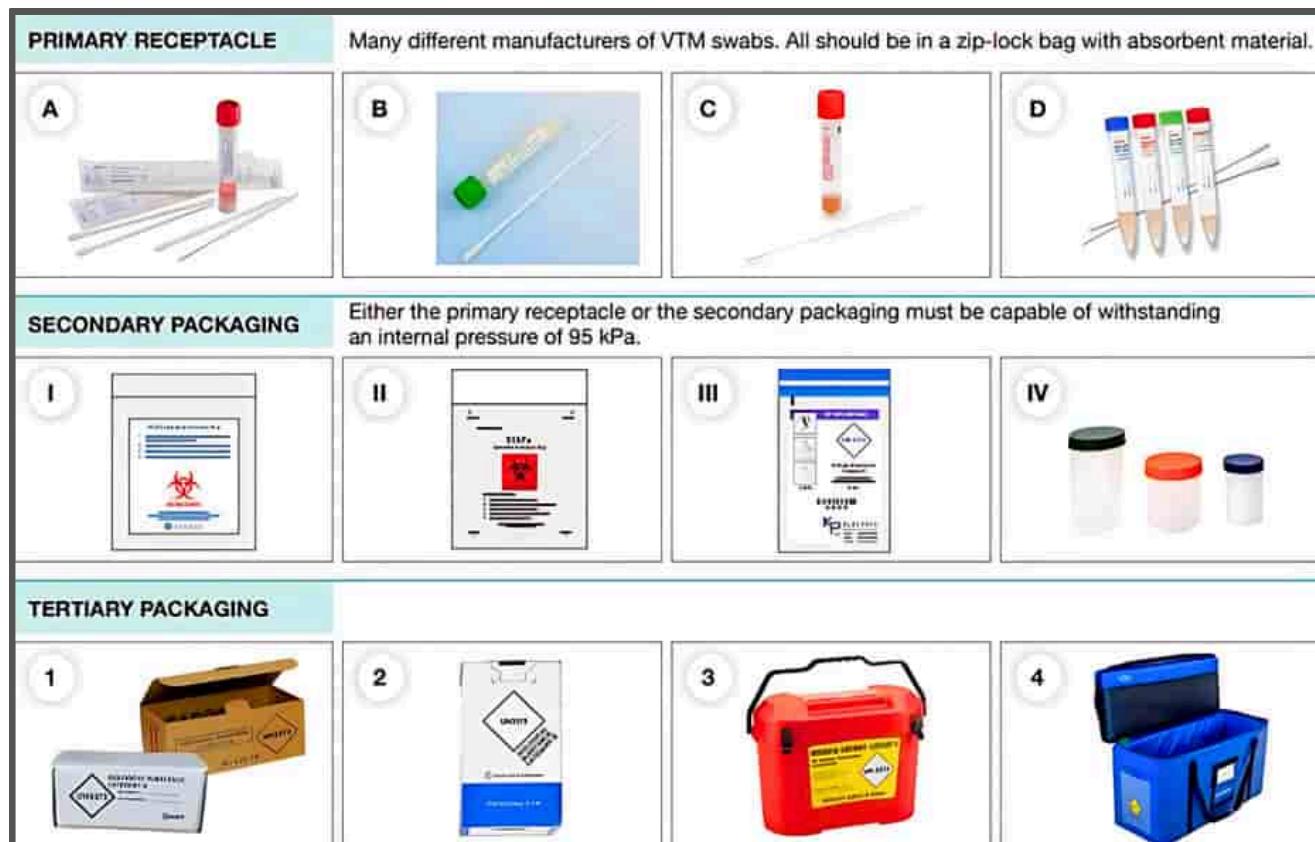
Viral transport media and Swabs



- Seal the neck of the sample vials using parafilm.
- Cover the sample vials using absorbent material.
- Arrange Primary container(vial)in secondary container and place it in zip lock pouch.
- Place the zip lock pouch inside a sturdy plastic container and seal the neck of the container.
- Using a thermacol box as an outer container and placing the secondary container within it, surrounded by hard frozen gel packs.
- Place the completes specimen referral form and request letter inside a leak proof, zip lock pouch.
- Secure the zip lock pouch with the specimen referral form on outer container.
- Attach the labels properly -Senders address, contact number, Biological substance category B and handle with care label.

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Packaging of swab after collection



SAMPLE REJECTION CRITERIA FOR COVID 19

- Dry swab
- Any tube that will lack labelling that includes media contents, lot and patient details will be rejected.
- Sample not received under proper storage conditions will be rejected. Unsealed sample container will be rejected.

SAMPLE ACCEPTANCE CRITERIA FOR COVID 19

- ICMR form must be received with sample
- Swab must be properly placed in VTM
- Sealed sample in ziplock pouch Non hemolyzed
- Proper temperature conditions while transporting sample
- Proper labelling on specimen container including name/age/gender (inner container) and other details like address/name /phone no mentioning "to be tested for COVID19 " on outer container.

SAMPLE STORAGE/SAMPLE STABILITY/RETENTION PERIOD

- Sample should be transported immediately to Preventive Health care according to SOP. If it not transported and processed immediately it should be refrigerated to 2°C to 8°C.
- Sample storage temperature recommended guidelines (ICMR GUIDELINES) as follows- Nasopharyngeal/oropharyngeal swab-
 - Transported at 4°C.
 - Till processing ≤5 days 4°C
 - If Processing >5 days--70°C.

Sample retention period - 1 month.

CYTOGENETIC

Classical Cytogenetic analysis and “Karyotyping” are the crucial diagnostical methods of Medical Genetics. Genetic diagnosis helps doctors in better case management and reduces emotional stress for patients. Hundreds of syndromes and health problems are caused by genetic defects. Cytogenetics helps in identifying these defects at the chromosomal level through genetic testing. The most common conditions when Karyotyping is opted are bad obstetric history, infertility, recurrent abortions, neonatal deaths, still birth, mental retardation, ambiguous genitalia, menstrual disorders, and developmental problems in children, congenital anomalies, abnormal ultrasound findings, advanced maternal age, etc.

A. Peripheral blood chromosomal analysis

- Take a Sodium-Heparin vacutainer (green-top). Check for date of expiry. Label the vacutainer with the name of the patient, sex, date and time of sample collection.
- Collect 3-4 ml of the whole blood (depending upon the capacity of the vacutainer.) 2ml of the sample is sufficient in case of neonates.
- Invert the vacutainer gently few times for thorough mixing.
- The samples collected should be transported to the laboratory at room temperature, sealed in a self-locking plastic bag to prevent cross contamination, placed in a Styrofoam box.
- Ensure that the sample reaches the laboratory at the earliest and within 24 hours of sample collection.
- In case the samples need to be kept overnight at the collection center, store the sample at 2-8°C and send it to the lab next day morning at the earliest. Do not draw blood from patients suffering from fever, cold or those on antibiotics.

B. Amniotic Fluid Collection

- A qualified and experienced professional, under completely aseptic conditions and ultrasound guidance, should only sample amniotic fluid.
- Take 2-3 sterile, clean, screw cap centrifuge tubes (capacity 15ml) and label with patient name and date of sample collection.
- Collect 20-30 ml of amniotic fluid.

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- Do not add any additives to the amniotic fluid. Do not heat or freeze the same.
- Transport the samples collected to the laboratory at room temperature sealed in a self-locking plastic bag to prevent cross contamination.
- Ensure that the sample reaches the main laboratory at the earliest and within 24 hours of sample collection.
- In case the samples need to be kept overnight at the collection centers, store the samples at 2-8°C and send it to the laboratory next day morning at the earliest.
- Amniotic fluid samples are more prone to bacterial contamination, acquired during transportation. Therefore, careful handling is a must during sample collection and transportation.

C. Fetal cord blood chromosomal analysis

- A qualified and experienced professional, under completely aseptic conditions and ultrasound guidance, should only sample cord blood.
- Take a Sodium-Heparin vacutainer (green-top). Check for date of expiry. Label the vacutainer with the patient, sex, date and time of sample collection.
- Collect 2-4ml of Cord Blood and gently transfer it to a sodium-Heparin vacutainer (green-top) for transport.
- Collect cord blood after 18th week of gestation.
- Invert the vacutainer gently few times for thorough mixing.
- The samples collected should be transported to the laboratory at room temperature, sealed in a self-locking plastic bag to prevent cross contamination. Ensure that the sample reaches the laboratory at the earliest and within 24 hours of sample collection.
- In case the samples need to be kept overnight at the collection center, store the samples at 2-8°C and send it to the lab next day morning at the earliest.

D. Fetal heart blood chromosomal analysis

- A qualified and experienced professional should only sample heart blood.
- Take a Sodium-Heparin vacutainer (green-top). Check for date of expiry. Label the vacutainer with the name of the patient, sex, date and time of sample collection.
- Collect 2-4 ml of heart blood and gently transfer it to a sodium-Heparin vacutainer (green-top) for transport.
- Collect cord blood after termination of a pregnancy only.
- Invert the vacutainer gently few times for thorough mixing.

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- The samples collected should be transported to the laboratory at room temperature, sealed in a self-locking plastic bag to prevent cross contamination, placed in a Styrofoam box.
- Ensure that the sample reaches the lab at the earliest and within 24 hours of sample collection.
- In case the samples need to be kept overnight at the collection center, store the samples at 2-8°C and send it to the lab next day morning at the earliest.

E. Bone marrow aspirate chromosomal analysis

- All bone marrow aspirates for chromosomal analysis should be collected in the media provided by the lab [RPM] media supplemented with fetal bovine serum, antibiotics and preservative free heparin] or in Sodium-Heparin.
- Label the sample collection vial with the name of the patient, sex, date and time of sample collection.
- Collect 3-4ml of the whole blood (depending upon the capacity of the vacutainer.) 2ml of the sample is sufficient in case of neonates.
- Invert the vacutainer gently few times for thorough mixing.
- The samples collected should be transported to the laboratory at room temperature, sealed in a self-locking plastic bag to prevent cross contamination.
- Ensure that the sample reaches the lab at the earliest and within 24 hours of sample collection.
- In case the samples need to be kept overnight at the collection center, store the sample at 2-8°C and send it to the lab next day morning at the earliest.
- Bone marrow aspirates generally tend to dry up during transportation, hence any bone marrow aspirate should reach the lab at the earliest. Any bone marrow aspirate which is dried up will be rejected.
- Kindly furnish the stage of cancer along with the clinical history.

F. Products of conception chromosomal analysis

- Always collect products of conception in normal saline.
- Transport the samples collected to the laboratory at room temperature sealed in a self-locking plastic bag to prevent cross contamination.
- Do not collect products of conception in Formalin.

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- Ensure that the sample reaches the lab at the earliest and within 24 hours of sample collection. In case the samples need to be kept overnight at the collection center, store the sample at 2-8°C and send it to the lab next day morning at the earliest.
- The container used for collection of products of conception should be a sterile container.
- Do not use urine containers for shipping.
- The possible causes of rejection of a POC sample are gross contamination, necrotic tissue, specimen in fixative, sample more than 24-48 hrs old and lack of suitable fetal material for culture.
- See Table below for details of this tissue of choice for appropriate sample for Products of Conception for chromosomal analysis (for a specific case history).

S.No	Case	Tissue of Choice	Alternative tissue	Volume/Qty of sample	Collection vial
1	Intrauterine fetal demise (IUFD)	Amniotic fluid	Chorionic villi	I. 20-30 ml of amniotic fluid. II. 1.0 mm of chorionic villi	Amniotic fluid-sterile tubes, 15 ml capacity, screw cap Chorionic villi- medium provided by the lab, in a sterile container.
2	Therapeutic abortions	Amniotic fluid	Chorionic villi	I. 20 – 30 ml of amniotic fluid. II. 1.0 mm of chorionic villi	Amniotic fluid-sterile tubes, 15 ml capacity, screw cap Chorionic villi- medium provided by the lab, in a sterile container.
3	Late pregnancies	Fetal cord blood	Fetal Skin	I. 3 ml of fetal cord blood II. 2 cm x 0.5cm is ideal of fetal skin	Cord blood – Sodium-Heparin vacutainer. Fetal skin – villi- medium provided by the lab, in a sterile container.

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4	Intrauterine fetal demise (IUFD)	Stillborn cases	-	A small piece of tissue with sub epidermal layers.	Placental villi-medium provided by the lab, in a sterile container.
5	Late Pregnancies	Fetal cord blood	Fetal heart blood	3 ml of fetal cord / heart blood	Cord/Heart blood should be collected in sodium-Heparin vacutainer. Blood from a cardiac stab often yields a good sample, as does cord blood.

Details of the tissue of choice for appropriate sample for Products of Conception for Chromosomal Analysis (for a specific case history).