

**A.J. HOSPITAL AND RESEARCH CENTRE
LABORATORY SERVICES**



**AJ HOSPITAL AND RESEARCH CENTRE
KUNTIKANA MANGALORE - 575004**

SAMPLE COLLECTION MANUAL

DOCUMENT NUMBER: LAB/QR/SCM-21



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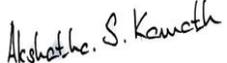
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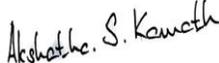
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C. ISSUE CONTROL

1. This section manual is released under the authority of the Lab Director, AJ HRC.
2. All the revisions to this section manual are to be reviewed and approved by the Lab Director.
3. Distribution of this manual is to be controlled by the Quality and technical manager and the original is to be retained by the Quality and technical manager.
4. The issue of each revised pages(s) is to be incremented and dated.
5. Details of all changes to be recorded in the amendment record.

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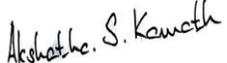
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D. AMENDMENT RECORD

SI. No	Section/Clause/Para/Line (As applicable)	Date of Amendment	Amendment made	Reasons of Amendment	Signature of Quality Manager	Signature Of Lab Director
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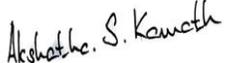
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E. INTRODUCTION

AJ Laboratory services, a part of parent organization AJ hospital and research Centre registered under Laxmi memorial educational trust was started in May 2019.

The laboratory is in the second floor B block of AJ hospital and research Centre. It occupies an area of 5000 sqft floor area. The laboratory is well equipped with equipment's of latest technology for accurate testing and provision of diagnostic services. The laboratory receives samples from OPD/IPD/Walk in and MHC patients. OP samples are collected in two sample collection area is located at ground floor OPD which is open from 6:00am to 7:00pm. The second sample collection area which is located in second floor 2B OPD (near to AJ Laboratory services) is open 24 x 7. The full laboratory conducts various test documented in Directory of services and scope under the discipline of Hematology, Clinical Pathology, Clinical Chemistry, Immunoassay, Microbiology and Infectious Disease Serology.

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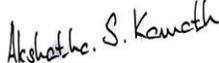
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1. PREPARING THE PATIENT FOR SAMPLE COLLECTION

Purpose: Adequate patient preparation, specimen collection, and specimen handling are essential prerequisites for accurate test results. Sample collection manual provides essential information on how to collect a proper quality & quantity of different samples required for the laboratory testing.

Responsibility: Phlebotomist (Laboratory technician/Nursing staff)

a) Patient Instructions

- It is important to gain patient's understanding and cooperation in obtaining an acceptable specimen.
- Provide the patient, in advance, with appropriate collection instructions and information on fasting, diet, and medication restrictions when indicated for the specific test.
- In case of HIV testing patient's consent is obtained by getting the Consent Form filled and signed by the patient after Counselling by a Healthcare professional.
- **Profile Testing:** Fasting or Diet Requirements
 - ✓ For Fasting Blood Sugar minimum of 8 hours fasting is recommended.
 - ✓ For Lipid profile testing, Laboratory suggests minimum of 10 to 12-hour fasting samples. (not more than 16 hours recommended)

b) Patient States

Basal State:

- In general, specimens for determining the concentration of body constituents should be collected when the patient is in a basal state (i.e., in the early morning after awakening and about 10-12 hours after the last ingestion of food). Reference intervals are most frequently based on specimens from this collection period.

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- The composition of blood is altered after meals by nutrients being absorbed into the bloodstream. Consequently, postprandial blood (blood drawn after a meal) is not suitable for some chemistry tests. An overnight fast is preferable to ensure that the patient is in the basal state. This minimizes the effects of ingested substances on the test results. Before collecting the specimen, ask the patient when he/she last ate or drank anything.
- If the patient has eaten recently and the physician wants the test to be performed anyway, it should be mentioned in the request form as “non-fasting and indicate the time the patient ate. **Fasting does include abstaining from coffee, tea, or sugar-free products.**
- Fasting or diet restrictions, such as low-fat diets, should be explained in detail, particularly to age or overanxious patients or their caregivers. Inform patients that fasting does not include abstaining from water. Dehydration resulting from water abstinence can alter the test results.

When specimens are not collected in the basal state, the following additional effects should be considered.

- **Exercise:** Moderate exercise can cause an increase in blood glucose, lactic acid, serum proteins, and creatinine kinase (CK) and Prolactin levels.
- **Emotional or Physical Stress:** The clinical status of the patient can cause variations in test results.
- **Time of Day of Collection.** Diurnal variations and variations in circadian rhythm can also affect test results.

For example: growth hormone peaks in the morning before waking and decreases throughout the day. Serum iron levels may change as much as 30% to 50%, depending on individual variation, from morning until evening.

Note: For chemistry profiles, 10 - 12 hour fasting specimens are recommended.

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c) Timed Specimens

There are two types of timed blood specimens: One is for a single blood specimen ordered to be drawn at a specific time. The other is for a test that may require multiple blood specimens to be collected at several specific times.

- **Single Specimens.** Here are some instances in which timed single specimens may be required.
 - Fasting plasma glucose alone or in conjunction with a random glucose determination, as recommended by the American Diabetes Association, to diagnose diabetes. Fasting here is defined as no caloric intake for at least eight hours.
 - Postprandial glucose may be performed two hours after a meal for a timed test that is helpful in diabetes detection.
 - Blood glucose determinations may be ordered at a specific time to check the effect of insulin treatment.
 - Blood cultures may be ordered for a specific time if a bloodstream bacterial infection is suspected.
 - Therapeutic monitoring of patients on medication. (Therapeutic drug monitoring (TDM) is the term used for measuring the level of some drugs at timed intervals as a way to determine the most effective dose or to avoid toxicity. Most drugs do not need to be monitored this way because your doctor can look for an improvement in symptoms or use tests like blood pressure, temperature, or blood glucose to determine if the dose is correct.) Reference: <https://www.labtestsonline.org.au/learning/test-index/thdm>
- **Multiple Specimens.** Here are some instances in which timed multiple specimen tests may be ordered.

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GCT :-

The most common timed procedure is a Glucose challenges test. The sample collected from the patient after Oral glucose and blood specimen are drawn at fixed intervals.

- For Glucose Challenges Test (GCT) 1 sample will be collected after 1 hour or 2hour after the oral glucose consumption (50 or 75 gms).

GTT:-

- The most common timed procedure is a glucose tolerance test. First, a blood specimen is drawn from a fasting patient. Then, the patient is given Oral glucose and blood specimens are drawn at fixed intervals.
- For Glucose Tolerance Test (GTT) 3 or 4 samples will be collected every half an hour or hourly after the oral glucose consumption (75 or 100 gms) followed by the fasting sample collection.
- To test the effect of a certain medication, a physician may order the same test to be obtained on consecutive days, before, during, and after the patient has received a medication.
- Collection of an acute and convalescent serum to aid in the diagnosis of a viral infection when culturing is not feasible.
- Other examples include such tests as occult blood, ova and parasites, and blood cultures.

d) . Sequential Sampling

Diagnosis of many endocrine diseases requires sequential sampling of blood and/or urine.

- All sequential specimens are from the same patient and are sent to the laboratory at the same time.
- The specimens are clearly labeled during the specimen collection with their chronological sequence (1 of 6, 2 of 6, or time drawn) and with the patient's name, other unique identifier, and date of collection.

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- Only one test request form accompanies the serial samples, and it is completed with all patient information, including any medications administered and the number of samples will be sent.
- The test request form will be sent with first sample of the sequential collection.

e) Serial Monitoring

- Monitoring a patient over time for a specific condition is a variation of sequential sampling. Many tumor markers (tests used to follow the patient's response to treatment for cancer) may be monitored over the course of several years.
- Specific instructions for serial monitoring are found in the test description for the applicable test being monitored.

Specimen Collection details for such tests is as follows:

❖ **ACTH STIMULATION TEST**

Basal Cortisol Levels: After synacthen injection (250 µg IV)

- 30 minutes' post injection Cortisol levels.
- 60 minutes' post injection Cortisol levels or as prescribed by clinician.

❖ **PROLACTIN /FSH/LH/TESTOSTERONE**

Tri pooled sample taken at 20 minutes' interval. Each sample to be labeled separately as

1. 0 min.
2. 20 min.
3. 40 min

❖ **GROWTH HORMONE TEST**

1. Basal level of Growth hormone.

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2. After 110 gms (equivalent to 100 gms of anhydrous glucose) of oral Glucose load 2nd sample for Growth Hormone estimation after 60 minutes.

❖ **INSULIN**

1. Fasting blood sugar – Insulin Sample
2. Post Glucose Blood Sugar 1 hr. Insulin
3. Post Glucose Blood Sugar 2 hrs. Insulin or as prescribed by clinician.

❖ **GLUCOSE TOLERANCE TEST (As per ADA-American Diabetic Association Guidelines)**

Fasting blood sugar

- 30 min.
- 60 min.
- 90 min.
- 120 min.

Pre-analytical Instructions

1. Following an overnight fast of minimum 8 to 10 hrs
2. Administer 82.5 gms (equivalent to 75 gms of anhydrous glucose) of oral Glucose (Glucon-D) dissolved in 400 ml of water.
3. Draw fasting blood sugar and blood sugar samples at 30, 60, 90 and 120 minutes after ingestion.

❖ **Glucose Tolerance Test (3 Samples)**

1. Fasting blood sugar
2. After 75 gms of oral Glucose
3. 60 min.
4. 120 min.

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Note:

- In pregnancy 110 gms of Glucose (equivalent to 100 gms of anhydrous Glucose to be administered)
- Collect the urine specimen for urine sugar test along with every blood sugar sample are drawn.

❖ **Post Glucose (50 gms- Glucose Challenge Test) - 1 hr**

- In all cases except for Post Glucose (50 gms) - 1 hr (pregnancy screening), patient is asked to come without fasting.
- After completing the front office procedures, the amount of glucose as requested is weighed prior and given in not more than 200 ml of water, after ensuring that all the glucose has dissolved. Specific instructions are given to the patient requesting minimal movement.
- Collect the blood sample exactly after one hour of intake of glucose.
- In all cases, request the patient to inform the doctor or technician, if he / she feel any discomfort, then the test procedure will be discontinued.

Note:

- In case doctor requested urine analysis or urine sugar test, collect the urine specimen prior to the consumption of oral glucose.
- In case clinician requests for post glucose after two hours. Then 75 gms of oral glucose to be given two hours prior to the blood collection.
- **For children up to 13years:** Requested for Post glucose testing, the amount of glucose is weighed as per the below calculation: Glucose quantity = 1.75 gms x weight of the child.

Interference of Medications and Other Substances

- Many common prescribed and non-prescribed (over-the-counter) medications can interfere with chemical determinations or alter levels of substances measured. Drug interference is complicated and often method-dependent such that only general recommendations can be stated here. Precautions to be observed must be determined by the physician, and the

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patient must then be told to avoid specified medications for the necessary periods of time prior to specimen collection.

- If the patient cannot be taken off the medication in question, its presence should be noted on the test request form.

Note: For practical purposes, unless drug interference can be avoided by ordering an alternate test method, drug therapy under supervision of the clinician may be discontinued for a period of 2-3 days and tests repeated, especially in cases where false abnormal (and occasionally false normal) findings are suspected.

Summary: Interference of Medications and Other Substances.

- Direct drug interference is least likely to occur in blood tests, as drug concentrations are usually very low; however, drugs or their metabolites frequently are concentrated in the urine in sufficient amounts to interfere significantly with urine assay.
- Drug interference of notable clinical significance has been well-documented in the following instances.
- Thiazide diuretic therapy. The pharmacologic or toxic effect is hyper-uricemia and hyperglycemia.
- Oral contraceptive causes a decrease in serum vitamin B12 levels that is in many cases indistinguishable from Vitamin B12 deficiency of any cause. They also cause an increase in total serum thyroxin-binding globulin. This results in an increase in both total serum thyroxin and unsaturated thyroxin-binding globulin but with no significant change in unbound (free) thyroxin.
- Many medications have been shown to have long-term residual effects that interfere with testing.

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2. PATIENT REGISTRATION & SERVICE BILLING FOR LAB INVESTIGATION

PURPOSE: The purpose of this policy is to describe the process of verifying the identification of a patient at each encounter & to ensure that all the tests done in laboratory are charged to the patient and are billed accordingly

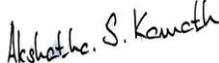
SCOPE/RESPONSIBILITY: It is the responsibility of the staff to properly identify all patients using two patient identifiers prior to any procedure/billing.

Key points:

- When patient comes to the Lab reception (Ground floor Lab reception & 2nd B Sample collection room) for query or Lab investigation purpose first and foremost thing to be done is greet the patient.
- Ask the patient to submit the test requisition form.
- Identify the patient by double verifying the UHID and patient name.
- After identification of patient go to the OP service billing and check the visit entry by typing the patient UHID in system.
- Make the fresh visit entry for each patient if visit entry is not available in system.

a) Visit entry:

- Go to front office module in HIS and click to visit entry.
- Type the patient UHID and verify before proceeding.
- Select the patient source (Walk-in or Diagnostic service)
- Select visit type as 'Service'
- Select the location as 'AJ Lab'

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- Select the department (eg: urology or endocrinology ect.,) and select the attending physician.
- Select the consultation type ‘Consultation OP - No Fees’ and tick the ‘free consultation’ check box
- Write the consultation remark as ‘AJ Lab’ and Save the visit entry
- Once the visit entry is done generate the service billing.

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b) Laboratory Service billing

- Once visit entry is done go to Billing module and select OP service billing
- Type the patient UHID and verify before proceeding.
- Details of the patient will be auto-filled by system.
- Double check the details and click the grid action to add the tests requested.
- Select the payment mode (card or cash)
- Collect the amount if cash payment
- Swipe the card and enter the card payment details in system and save the bill.

The screenshot shows the 'OP Service Billing' interface. It includes a search bar at the top, a left-hand navigation menu with categories like 'OP Billing', 'IP Billing', 'Corporate Transactions', and 'ER Billing'. The main form contains fields for 'Bill No.*', 'UHID*', 'OP No*', 'Patient Name', 'Credit Available', 'Loyalty Card', 'Corporate', 'Attending Physician', 'Bill Type*' (set to 'Cash'), 'Price List*', 'Concession Source', 'Concession Referred By', 'Authorized By', and 'Blood Group'. Below these is a 'Service Particulars' section with a 'Grid Actions' button and a table with columns: S.No, Service, Doctor Name, Rate, L. Discount, Cons. Type, Cons. Rate, Net Amount, and Remarks. A 'Payment' section shows 'Concession Amt.', 'Net Amount', and 'Payment Mode*' (set to 'Cash'). A 'Remarks' text area is at the bottom.

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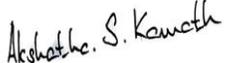
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In case of OSP patient:

- Generate the OSP bill if the patient came for only lab investigation or if patient does not have any UHID.
- Go to Billing module and select OSP service billing
- Fill the patient details in the system (Name, Gender, Age, Mobile number & referred by Doctor name)
- Double check the details and click the grid action to add the tests requested.
- Select the payment mode (card or cash) and save the bill.
- Collect the amount if cash payment.
- Swipe the card and enter the card payment details in system and save the bill.

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Once the Billing is completed direct the patient with Test Request Form (TRF) and Bill invoice for Sample collection procedure.

In case of In-Patient, it is pre requisite & mandatory to fill the online request for the tests requested by treating doctors at the ward/ICU's/Emergency department. The charges of lab investigation are added in IP billing.

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3. SAMPLE BAR CODE LABELING

- Once the billing is completed, the HIS system will generate unique Lab sample number.
- To take the print of lab barcode go to laboratory module and select Sample collection.
- Type the UHID of the patient and click to the sample order number.
- Tick the box of tests which sample needs to be collected and click the ‘collect’ option.
- Print the barcode by pressing ‘print barcode’
- Affix the barcode on the tubes in which sample needs to be collected.
- Double verify the UHID and patient name in request form as well as in barcode.
- A good practice of barcode labelling is to apply barcode before patient leaves the sample collection area (or at the bedside when collecting samples in ward) to prevent wrong labelling.
- Collect the patient sample as per the Sample Collection Manual (LAB/QR/SCM-21) and transport the sample to the 2B laboratory immediately as possible.
- Once the samples are physically received in 2B laboratory accession area, the samples are acknowledged in HIS-Lab module.
- In case of IP samples received from ward, apply the label with sample number after acknowledgement in system in such a way that the original label on the tube can also be viewed.

a) BENEFITS of barcode labelling:

- Reduces the patient/sample identification errors if applied properly.
- It helps to search the patient previous result and to cross check the patient details.
- Auto interface of reports from lab equipment to HIS.

Note: Automated data entry is highly accurate, which improves patient safety by eliminating errors. As a side benefit, barcode sample identification and data entry also

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saves time, enabling lab staff to spend more time on clinical rather than clerical activities.

b) When sticking labels on sample tubes, MAKE SURE

- Barcode is aligned straight for lab equipment barcode scanners to read.
- Check the quality of barcode specially for the visibility of Unique identifiers on the barcode.
 - Patient Name
 - Patient lab Number and barcode
- Leave a visible window to allow laboratory personnel to check
- Do not handwrite on barcode labels.

c) UNACCEPTABLE EXAMPLES OF SPECIMEN LABELLINGS

- Barcode position too low
- Barcode label over the cap
- 2 barcode labels on 1 tube
- Barcode in wrong direction
- No barcode label on tube
- Many tubes sharing 1 barcode labels
- These types of specimen labelling are not accepted by our Laboratory Automation System. (unacceptable samples will be rejected by lab professionals)
- Manual intervention is required to reprint the appropriate labels
- Causes increased result turnaround time
- Results in a longer waiting time for the patient = Unsatisfactory experience for patient.

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d) Segregation of the specimen

1. Segregate the samples, department wise in separate racks.
2. Once labeled, send the sample rack to the concerned department and document the receipt of sample received by respective sections in the Excel sheet maintained as a soft copy in the Computer system of reception area

Any test discrepancy in test request & / or specimen is treated as unacceptable specimen for want of further clarification or rectification. Rejection of these samples with reasons are listed in HIS and informed to respective departments over the phone and a repeat sample is requested by the technical staff of the lab to the Nurse in-charge of respective patients. All specimens & test request must be handled as per the Sample Collection Manual.

e) SPECIMEN CHECKING

The total number of specimens must be same as mentioned on the requisition form. They sign off after the total numbers of specimens are confirmed. If the actual number of specimen is not matching to the number expected i.e. if extra / less specimens received, then the technician investigates the discrepancy with same.

Any STAT (instantly” or “immediately.”) test [Analyses listed in STAT list] requested must be handled on priority in the following manner

- The **STAT** specimens (red sticker label) must be checked for suitability & registered immediately.
- The barcode must be affixed & specimens sent to the concerned testing sections
- These **STAT** specimens are immediately processed

All other specimens are then checked for suitability with respect to identity of the patient; traceable by request form, type, quality, quantity and received in appropriate condition for the test requested. Suitable specimens are those, which satisfy all conditions with the complete test requisition i.e.

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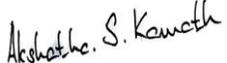
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without any discrepancy in patient's details, specimen details, & clinical history including information on therapy with Cytotoxic drugs. The identity of the sample-receiving technician is recorded on the requisition form

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4. LABORATORY COLLECTION OF BLOOD SAMPLES AND TRANSPORTAION

Purpose: Collection and Handling of Blood Samples

Scope: To define the blood collection procedures for all sections including Hematology, Clinical pathology, Biochemistry, Immunoassay, Microbiology and Serology

Responsibility: Lab Director/ Laboratory Consultants/lab Q & T manager/ Deputy Q & T mangager/Technical staff

Procedure: as under

a) Blood specimen collection and handling

- All the request forms shall be checked for completion prior to sample collection
- For specimens other than blood, appropriate containers should be labeled and given to the patients with proper instructions for collection and handling of specimens.
- For blood collection, the patients are directed to sample collection room/phlebotomy area

b) Approaches and Identify the Patient

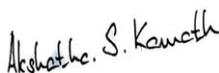
1. Out Patient Sample Collection

- The technician should identify himself or herself, explain the procedure to the patient and gain the patient's confidence. The technician must NOT perform blood collection against the patient's or guardian's verbal consent.

2. In Patient Sample Collection

- The doctor / paramedical staff / nurse/technicians responsible for collection of sample. He/she shall identify himself or herself and gain the patient's confidence.

3. Identify Patient

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- Identification of the patient is crucial. The technician must ensure that the blood specimen is being drawn from the individual designated on the request form. The following steps are a suggested for ensuring patient identification regardless of the clinical setting.

c) Patient who is Conscious

The steps are as follows:

1. Ask the patients to give out full name, address and identification number (Hospital number)
2. Compare this information with the information on the request form.
3. Ask in patients for the same information and compare this information with the patient's request form / chart.
4. In case of any discrepancy, report to the senior / In charge / doctor for clarification.

d) Patient who is Semiconscious, Comatose or Sleeping (In patient)

- The person responsible for sample collection must take special care when drawing blood from semiconscious, comatose or sleeping patients to anticipate any unexpected movements of jerks either while introducing the needle while it is in place in the arm.
- Sleeping patients should be awakened before drawing blood.

e) Patient who is Unconscious(Too Young, Mentally Incompetent, or does not speak the Language of the Person Responsible for Sample Collection.)

In any of these circumstances, the following steps are suggested:

1. Ask the relative or a friend to identify the patient by name, address and hospital number.
2. Compare the data with the information on the patient's chart or the request form.
3. In case of any discrepancy, report to the senior / In charge / doctor for clarification.

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f) Procedure for Identifying Unidentified Emergency Patients.

The patient must be positively identified when the blood specimen is collected. The unidentified emergency patient should be given temporary but clear designation until positive identification can be made.

g) Verify patient Diet Restrictions

- Some tests require the patient to fast and / or eliminate certain foods from the diet before the blood drawn. Time and diet restrictions vary according to the test. Such restrictions are necessary to ensure accurate test results.

h) Assemble Supplies

The following supplies should be available at any location where Venipuncture is performed routinely:

- Blood Collection tubes / blood culture bottles
- Needle 22G
- Single-use tube / needle holder
- Syringe (2ml,5ml and 10ml)
- A tourniquet
- Alcohol swab
- Adhesive ANTISEPTIC bandages
- Gloves
- Sharps Disposal Container (Puncture proof container marked “Biohazardous”.)
- Dry cotton
- Dustbin as per colour code

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i) Position Patient

1. Procedure for seating patient

- Ask the patient to be seated comfortably in a chair suitable for Venipuncture. The chairs should have arms to provide support and prevent falls, if the patient loses consciousness.
- Have the patient position his / her arm rested in a comfortable position and extend the arm to form guide the patient to keep his/her a straight line from the shoulder to the wrist. Arm should not be significantly bent at the elbow.

2. Procedure for patient lying supine

- Ask the patient to lie down on his / her back in a comfortable position.
- If additional support is needed, place a pillow under the arm from which the specimen is being drawn.
- Have the patient position his / her arm extends to form a straight line from the shoulder to the wrist.

j) Apply Tourniquet

A tourniquet is used to increase venous filling. This makes the vein more prominent and easier to enter.

1. Precautions while using tourniquet

- Tourniquet application should not exceed one minute as localized stasis with haemo concentration and infiltration of blood into tissue can occur. If the patient has a skin problem, the tourniquet should be applied over the patient's gown or a piece of gauze pad or paper tissue should be used so that the skin is not pinched.

2. Tourniquet location

- Wrap the tourniquet around the arm 3 to 4 inches above the Venipuncture site.

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3. Blood Pressure cuff

- If a blood pressure cuff is used as a tourniquet, inflate it to 40mm Hg.

4. Ensure patient's hand is closed

- The veins become more prominent and easier to enter when the patient forms a fist. There must not be vigorous hand exercise. Vigorous hand pumping can cause changes in the concentration of certain analytes in the blood.

5. Select Vein

- Median cubital and cephalic veins are used most frequently. Veins on the back of the hand also can be used. Veins on the underside of the wrist must not be used

6. Factors to avoid in site selection

- **Extensive scarring from burns and surgery** - it is difficult to puncture the scar tissue and obtain a specimen.
- **Mastectomy**
A physician must be consulted before drawing blood from the side on which a mastectomy was performed because of the potential for complications due to lymphostasis
- **Hematoma** - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- **Intravenous therapy (I.V)** / blood transfusions fluid may dilute the specimen, so collect from the opposite arm if possible.
- **Cannula/catheter/fistula/heparin lock** - blood should not be drawn from an arm with a fistula or cannula.
- **Edematous extremities** - tissue fluid accumulation alters test results.

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The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.

Precaution for coagulation profile:

- Sample should never be collected from central venous catheter (CVC) line, Dialysis catheter and I.V. Cannulas.
- Quickly and vigorously pulling the syringe plunger while drawing blood or pushing the blood vigorously into the vacutainer and vigorously mixing the vacutainer must be avoided.
- In the following cases, the laboratory must be informed at all times:
 - Sample from the above mentioned or any deviation in collection procedure.
 - Anticoagulants or blood thinners on-flow.
 - Drug that influence the coagulation profile.
 - Difficulty in vein location, prolonged tourniquet application or milking the sampling site.

7. Procedure for Vein selection

- Palpate and trace the path of vein with the index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall.
- Thrombosed veins lack resilience, feel cord-like, roll easily, and should not be used.
- A tourniquet must be used to aid in the selection of a vein site unless specific tests do not require tourniquets (e.g. lactate, coagulation profile).
- The tourniquet should be released the moment blood starts flowing.
- If a tourniquet must be applied for the preliminary vein selection, it should be released and reapplied after two minutes.

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k) Put on Gloves

The phlebotomist must wear gloves (sterile/unsterile as per the availability) before the Venipuncture is performed.

l) Cleanse Venipuncture Site

The puncture site must be cleansed

1. Cleansing Method for Venipuncture

- Use a gauze pad/swab with 70% isopropyl alcohol solution.
- Cleanse the site with a circular motion from the center to the periphery.
- Allow the area to air dry to prevent hemolysis of the specimen and to prevent the patient from experiencing a burning sensation when the Venipuncture is performed.

2. For Blood Culture Collection

- For blood cultures, it is necessary to carefully disinfect the Venipuncture site.
- Chlorhexidine gluconate is recommended for infants two months and older and patients with iodine sensitivity.
- Cleanse the site with 70% alcohol, then swab concentrically, starting at the middle of the site with a 1 to 10% povidone-iodine solution (0.1 to 1% available iodine) / or chlorhexidine gluconate.
- Allow the site to air dry and then remove the iodine or chlorhexidine from the skin with alcohol.
- When specimens are obtained for blood cultures, disinfect the culture bottle stopper according to the manufacturer's instructions.

3. Touching the Site after Cleansing

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If the Venipuncture proves difficult and the vein must be touched again to draw blood, the site should be cleansed again.

m) Order of Draw

1. Plastic Venous Blood Collection Tubes

The following order-of-draw is recommended when drawing multiple specimens for clinical laboratory testing during a single Venipuncture. Its purpose is to avoid possible test result error due to cross contamination from additives. This procedure should be followed for venous blood collection tubes. The tubes should be mixed by inversion gently.

Proceed with the collection in the following “Order of Draw”

ORDER OF DRAW			
SL.No	Tube type	Reason	No. of inversion
1.	Blood culture tube	Sterile sample	8 - 10
2.	Citrate tube	To avoid mixing with other anticoagulant/ clot activator.	3 - 4
3	Red tube with or without clot activator, yellow tube with or without gel separator	Will enhance the clotting (Note: For adequate clotting keep the sample tube in upright position for 30 mins)	5
4.	Heparin tube with or without gel plasma separator	It is natural anticoagulant	8-10
5.	EDTA	Best calcium chelator, It preserve the cell morphology	8-10

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6.	Glycolytic inhibitor (Fluoride)	Sodium EDTA is an anticoagulant and Sodium Fluoride is as preservative, this Preserve Glucose value for another 48hrs.	8 - 10
7.	Microtainer (RED, EDTA)	Will enhance the clotting (Note: For adequate clotting keep the sample tube in upright position for 30 mins)- Plain Best calcium chelator, It preserve the cell morphology-EDTA	

Note: When using a winged blood collection set for veni-puncture 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 collecting 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 non-additive or a coagulation tube

2. Coagulation Testing

Studies have shown that the PT (INR) and APTT results are not affected if tested on the first tube drawn. If the volume is found insufficient, it may be advisable to draw a second tube for other coagulation assays. When a syringe system is used and a large specimen is taken, part of the blood from the second syringe should be used for the coagulation specimen. In the case of any unexplained abnormal coagulation test result, a new specimen should be obtained and the test repeated.

n) Perform Venipuncture

There are several different blood collections systems available that collect blood samples using different principles.

- Closed system refer to the exposure of the samples to a bare minimum thus prevent, contamination, spillage or evaporation.

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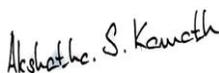


- Vacutainer are being used for blood collection with one-time exposure of blood at the site of prick.
- No spillage during transportation
- Processing of sample (centrifugation, separation of plasma, serum) is without any exposure of blood.

1. Venipuncture Procedure: using closed system of sample collection (Using vacuum blood collection tubes)

Procedure:

- Thread the appropriate needle into the holder until it is secure.
- When drawing blood for cultures, wipe the stopper with a suitable antiseptic solution. Make certain the stopper is dry before performing the Venipuncture.
- Make sure the patient's arm or other Venipuncture site is in a downward position to prevent reflux of "backflow".
- Hold the patient's arm firmly distal to the intended puncture site. The phlebotomist's thumb should be used to draw the skin taut. The thumb should be 1 to 2 inches (2.5 to 5.0 cm) below the Venipuncture site.
- To prepare the patient, inform him or her that the Venipuncture is about to occur.
- With the bevel up, puncture the vein with the needle at an angle of insertion of 30 degrees or less. Keeping the needle as stable as possible in the vein, push/connect the first tube onto the needle. Maintain the tube below the site when the needle is in the vein.
- Release the tourniquet as soon as possible after the blood begins to flow. Do not change the position of the tube until it is removed from the needle.
- Allow the tube to fill until the vacuum is exhausted and blood flow ceases. For tubes that contain additives, this will ensure there is a correct ratio of blood to additive.

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- When the blood ceases to flow, remove/disconnect the tube from the needle/holder. The sleeve re-covers the needlepoint that pierces the tube closure, stopping blood flow until the next tube is inserted / connected to the needle/holder. To obtain additional specimens, insert/connect the next tube to the needle/holder prior to withdrawing the needle from the vein. If only one tube is collected this must be removed prior to withdrawing the needle from the vein.
- Immediately after drawing each tube that contains an additive, mix the blood gently and thoroughly by inverting the tube according to the table To avoid hemolysis, do not mix vigorously.

2. Venipuncture Procedure Using Needle and Syringe

In general, Venipuncture using a syringe should be avoided for safety reasons. In case of difficult collections by needle / holder and vacuum blood collection tubes, Venipuncture procedure can be performed using a syringe draw; the following procedure is recommended.

- Assemble the needle and syringe.
- Hold the patient's arm firmly distal to the intended puncture site. The technician thumb should be used to draw the skin taut. This anchors the vein. The technician thumb should be 1 or 2 inches below the Venipuncture site.
- Prepare the patient by informing him or her that the Venipuncture is about to occur.
- With the bevel up, puncture the vein with the needle at an angle of insertion of 30 degrees or less.
- Keeping the needle as stable as possible in the vein, slowly withdraw the desired amount of blood.
- Release the tourniquet as soon as possible, after the blood begins to flow.

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- **Fill the Tubes If Syringe and Needle Are Used**

Syringe method of drawing venous blood is not recommended since it is much safer and easier to use a closed, venous blood collection tube system. If it is necessary to use a syringe, proceed with the following recommendations to transfer the blood from a syringe to a blood collection tube.

- Use the same “order of draw” as for a venous blood collection tube system.
- Mix additive tubes by inversion.

Tips for Successful Venipuncture When Using Hand Veins

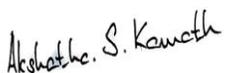
Hand position: When collecting blood from a hand vein, it is best practice to position the patient's hand slightly downward with the top of the hand facing you. The fingers of the patient's hand should be rolled underneath, forming a loose fist. Use your thumb to pull back gently on the surface of the skin, making the skin tight. The vein should be anchored adequately to proceed with venipuncture. The hand veins will be more prominent if the patient grips a pillow or a rolled up washcloth.

Tourniquet Position: The tourniquet should always be applied 3 - 4 inches above the needle insertion point. Therefore, when assessing for a usable vein in a hand, apply the tourniquet 1 - 2 inches above the wrist. If the tourniquet is on longer than one minute, release and reapply prior to venipuncture to avoid hemoconcentration.

Cautions:

Choose a straight section of the hand vein-- avoid the "intersection" or "V" where a vein branches into another vein. This juncture may contain a valve and could be damaged if punctured.

Only use the top of a hand for puncture. Veins on the palmar surface of the wrist, the fingers, and the lateral wrist above the thumb to the mid-forearm must not be used according to the 2017 CLSI

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standards. This will prevent the inadvertent puncture of hidden arteries, tendons, or nerves in the area.

**• Blood Specimen That Cannot Be Obtained (TROUBLESHOOTING GUIDELINES)
When a blood specimen cannot be obtained, it may be necessary to:**

- Change the position of the needle. If the needle has penetrated too far into the vein, pull it back a bit. If it has not penetrated far enough, advance, it farther into the vein. Rotate the needle half a turn. Lateral needle relocation should never be attempted in an effort the basilica vein, since nerves and the brachial artery are in close proximity.
- Try another tube to ensure the tube selected is not defective.
- Avoid manipulation other than that recommended. Probing is painful to the patient. In most cases another puncture in a site below the first site, or use of another vein on the other arm, is advisable.
- It is not advisable to attempt a Venipuncture more than twice. If possible, have another person attempt to draw the specimen or notify the physician.

Blood sample collection from the CVP Lines

- Stop the Intravenous line
- Tie a tourniquet above the IV line if it is in the limbs.
- Wait for at least 2 minutes, than with all strict aseptic precautions
- Draw 3 to 5 ml blood in a syringe and discard.
- Follow “order of draw” and take the sample.

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- **Ensure Patient's Hand Is Open- after sample collection**

Opening the patient's hand reduces the amount of venous pressure as muscles relax. The patient must not have allowed to pump the hand due to which bleeding can occur from venipuncture site.

- o) **Releases the Tourniquet**

Release the tourniquet as soon as possible after the blood begins to flow

- p) **Place the Gauze Pad/Swab**

A clean gauze pad/swab should be placed lightly over the Venipuncture site. Cotton balls may be used but ideally it is not preferred because of the possibility of dislodging the platelet plug at the Venipuncture site.

- q) **Remove and Dispose of the Needle**

Remove the needle and activate the safety mechanism according to the device manufacturer's instructions. Safely dispose of the unit into an easily accessible sharps container.

- r) **Bandages the Arm**

- **Under normal conditions:**

1. Place the gauze pad / cotton ball over the site, continuing mild pressure.
2. Check that bleeding has ceased, observe for hematoma.
3. An adhesive or gauze bandage is placed over the vein puncture site. It is recommended that hypoallergenic adhesive be available.

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• **Continued Bleeding :**

1. Watch for excessive bleeding.
2. If a hematoma develops observe for bleeding persists longer than five minutes, a nurse/phlebotomist should be alerted so that the attending physician can be notified. Pressure, applied with a gauze pad, must continue at the site as long as necessary to stop the bleeding.
3. Wrap a gauze bandage tightly around the arm to keep the pad in place tell the patient to leave the bandage on the site for at least 15 minutes.

• **Post puncture precaution :**

1. Observe the patient and ensure that bleeding from puncture site has stopped.
2. If the patient faints keep the head low with legs kept at higher level. Ensure adequate ventilation. Offer the patient water / glucose water.
3. If thrombosis / hematoma occurs at site use thrombophob gel for application at the site.

If Patient Fainting:

- Rarely, patients will faint during venipuncture.
- It is therefore important that patients are properly seated or lying in such a way during venipuncture so that if they do faint, they won't hurt themselves.

What to do?

- Immediately remove the tourniquet and needle from the patients arm, apply gauze and pressure to the skin puncture site.
- Call for help.
- If the patient is seated, place his head between his knees.
- If the patient is lying in bed, place the legs slightly elevated from body.

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➤ A cold compress on the back of the neck may help to revive the patient more quickly.

s) Label Blood Collection Tubes and Record Time of Collection

The patient and the patient's blood specimen must be positively identified at the time of collection. Blood specimens must be obtained in tubes identified with a label bearing at least the following.

- The patient's first and last name
- Age/sex
- Unique hospital identification number(UHID)
- The date
- The time (as required e.g. therapeutic monitoring)
- The completed label must be attached to the tube before leaving the side of the patient, and there must be mechanism to identify the person who drew the blood. Alternatively, the manufacturer's tube label can be inscribed with the patient's complete information.
- The laboratory documents the time when the specimen was collected. Whenever possible, a small signature or initials of the personnel responsible for collecting the specimen shall be recorded.

t) Transportation of primary sample with specification about time frame, temperature and carrier

1. From IPD wards / floors to Laboratory accession area

- The samples are collected by the TRAINED PERSONNEL

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- The sample are transported by the lab attender in the insulated box at the ward and bring it to the lab within 30 minutes.
- Once the sample reaches, the lab staff receives the sample. Compare the sample & online request by matching in every aspect.
- Examine the sample visually to evaluate for acceptability.
- Acknowledge the sample and apply the barcode for each samples.

Specimens that need transport at cool temperature

Certain tests require that blood specimens need to be transported with ice pack immediately following the Venipuncture, tests like:

- ✓ Ammonia
- ✓ Lactic acid
- ✓ Blood gas analysis
- ✓ Parathyroid (PTH) hormone etc...

2. From OP and Health lounge samples transportation:

- The samples are collected and properly marked with the patient name and UHID (or lab sample number).
- The sample will be sent along with test request form after sample collection.
- All sample are transported in insulated box to 2B lab immediately after the collection **(emergency sample are prioritized)**
- Examine the sample visually to evaluate for acceptability.
- The sample collection time shall be written in all test request form and the same shall be cross checked prior to acknowledgement of samples in lab.
- The specimen that needs to be processed immediately and for urgent report, they are sent to lab as soon as they are collected.

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- Delayed transportation after collection of sample may affect the result values.
 1. **Specimens that need transport at cool temperature**
Certain tests require that blood specimens need to be transported with ice pack immediately following the Venipuncture, tests like:
 - Ammonia
 - Lactic acid
 - Blood gas analysis
 - Parathyroid (PTH) hormone etc...
 - r) **Rejection of primary samples :**
 - Mislabeled /unlabeled specimens
 - Improper container
 - Quantity not sufficient for testing
 - Without test request
 - Hemolysis
 - Clotted
 - Samples not adhering to the vacuum blood collection tubes specifications
 - s) **Receipt primary sample in Accession area**
 1. **To receive a complete Test requisition form:**
 - ✓ Name of the physician or other person legally authorized to make request for examination
 - ✓ Type of primary sample and the anatomic site of origin.
 - ✓ Examination requested.
 - ✓ Clinical information of the patient for interpretation purpose
 - ✓ Date and time of sample collected.
 - ✓ Date and time of receipt of sample in the laboratory

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t) Unique identification of the patient

TO BE GIVEN BY LAB PERSONNEL as per the individual sections (to define it as per their requirement)

All the samples are barcoded after collection with relevant patient information (i.e. patient name, UHID, collection date and time etc..)

u) Receipt sample in case of urgent sample request

Criteria for urgency/emergency must be defined at individually.

- The samples which are marked with word “Urgent” on patient requisition forms or sticker “EMERGENCY”(red in color) stick on to samples are sent to the lab reception on urgent basis from both wards and OPD collection room by ward or lab attenders.
- This information for urgent samples may also be communicated telephonically the reception and respective sections of lab and also in on line test request shows red color sign in HIS/LHS.
- The specimens are checked for the proper identification as per SCM Rejection of primary samples
- Once the specimen is found OK it is allotted a unique identification lab number

1.4.20.5 Then the samples are specially transported to the respective sections of the lab on priority basis by accession technical staffs.

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5. PEDIATRIC AND NEONATAL BLOOD SAMPLE COLLECTON:

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.
- Venipuncture 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 pediatric nurse or the physician may need to draw the specimen. When a capillary blood specimen from a finger-prick or a heel-prick is appropriate. The blood from a capillary specimen is similar to an arterial specimen in oxygen content, and is suitable for only a limited number of tests because of its higher likelihood of contamination with skin flora and smaller total volume.

Practical guidance on pediatric and neonatal blood sampling

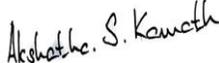
❖ Patient identification

- Use a wrist or foot band only if it is attached to the patient; DO NOT use the bed number or a wrist band that is attached to the bed or cot.
- If a parent or legal guardian is present, ask that person for the child's first and last names.
- Check that the name, date of birth and hospital or file number are written on the laboratory form, and match them to the identity of the patient.

❖ Venipuncture

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

Equipment and supplies for pediatric patients:

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Use a winged steel needle, preferably 21 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 hemolysis.
- 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) this helps to avoid collapse of the vein and may decrease haemolysis.
- gauze or cotton-wool ball to be applied over puncture site,
- laboratory specimen labels
- Well-fitting, non-sterile gloves
- a tourniquet
- 70% alcohol swabs
- Puncture-resistant sharps container.

❖ **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.**

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- Designate one phlebotomist as the technician, and another phlebotomist or a parent to immobilize the child.
 - 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”).

❖ **Drawing blood procedure:**

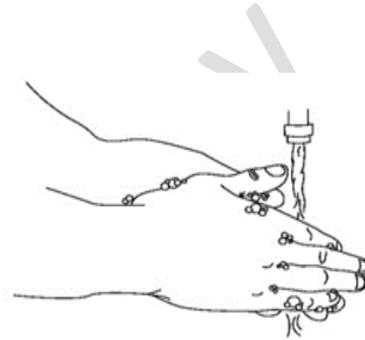
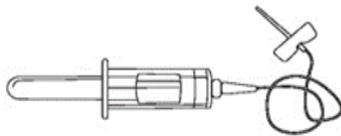
- Assemble equipment
- Select the site:
 - Extend the patient's arm and inspect the antecubital fossa or forearm.
 - Locate a vein of a good size that is visible, straight and clear.
 - Preferably warm the area of puncture with warm cloths to help dilate the blood vessels.
 - Use a trans illuminator or pocket pen light to display the dorsal hand veins and the veins of the antecubital fossa.
- Perform hand hygiene and put on gloves
- Disinfect the puncture site: 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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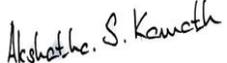
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1. Use a winged steel needle, usually 23 or 25 gauge, with an extension tube (butterfly). Keep the tube and needle separate until the needle is in the vein.
2. Collect supplies and equipment.
3. Perform hand hygiene (if using soap and water, dry hands with single-use towels).

CONTROL

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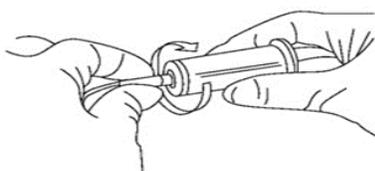
4. Immobilize the baby or child.



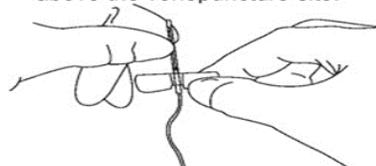
5. Put the tourniquet on the patient about two finger widths above the venepuncture site.



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



7. Attach the end of the winged infusion set to the end of the vacuum tube and insert the collection tube into the holder until the tube reaches the needle.



8. Remove the plastic sleeve from the end of the butterfly.



9. Disinfect the collection site and allow to dry.



10. Use a thumb to draw the skin tight, about two finger widths below the venepuncture site.



11. Push the vacuum tube completely onto the needle.



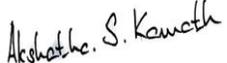
12. Blood should begin to flow into the tube.



13. Fill the tube until it is full or until the vacuum is exhausted; if filling multiple tubes, carefully remove the full tube and replace with another tube, taking care not to move the needle in the vein.



14. After the required amount of blood has been collected, release the tourniquet.

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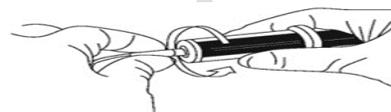
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15. Place dry gauze over the venipuncture site and slowly withdraw the needle.



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.



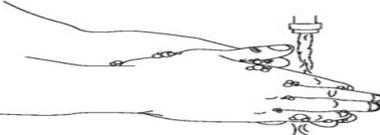
19. Properly dispose of all contaminated supplies.



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).

❖ **Finger and heel-prick**

- Whether to select a finger-prick or a heel-prick will depend on the age and weight of the child. Which procedure to select, based on these two elements.
- Patient immobilization is crucial to the safety of the pediatric and neonatal patient undergoing phlebotomy, and to the success of the procedure.
- A helper is essential for properly immobilizing the patient for venipuncture or finger-prick,

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1. Finger prick Procedure:

- The best locations for finger stick are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand.
- Do not use the tip of the finger or the center of the finger.
- Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface.
- The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
- When a site is selected, put on gloves, and cleanse the selected puncture area.
- Gently massage the finger toward the selected site prior to the puncture.
- Using a sterile safety lancet, make a skin puncture
- The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or “milking” that may squeeze tissue fluid into the drop of blood.
- Recap the tubes and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside(in case of IP)

2. Heel prick Procedure (infants):

The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates the proper area to use for heel punctures for blood collection.

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- Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
- Hold the baby's foot firmly to avoid sudden movement.
- Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
- Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
- Fill the required microtainer(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
- Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
- Remove your gloves and wash your hands

❖ **Order of draw**

With skin punctures, the hematology specimen is collected first, followed by the chemistry and blood bank specimens. This order of drawing is essential to minimize the effects of platelet clumping. The order used for skin punctures is the reverse of that used for venipuncture collection. If more than two specimens are needed, venipuncture may provide more accurate laboratory results.

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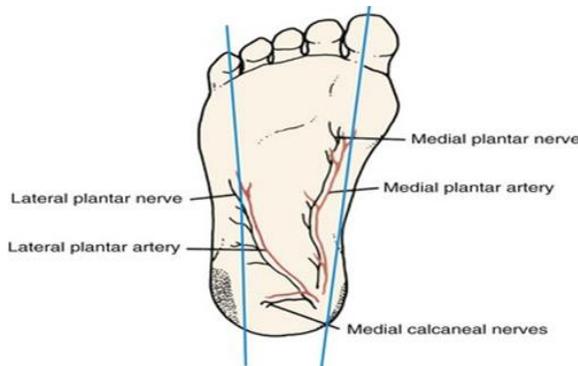
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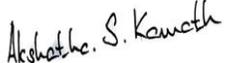
❖ **Unsuccessful attempts in pediatric patients**

Adhere strictly to a limit on the number of times a pediatric patient may be stuck. If no satisfactory sample has been collected after two attempts, seek a second opinion to decide whether to make a further attempt, or cancel the tests.



x) Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with draw.

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- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen

y) Blood Sample Handling and Processing:

Pre-centrifugation Handling: Pre-centrifugation Handling is the first critical step in the lab testing process, Specimen integrity can be maintained by following some basic handling processes:

- Fill the tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Tubes should be stored at 4-25°C (39-77°F) (prior to sample collection storage).
- Tubes should not be used beyond the designated expiration date.
- Mix all gel barrier and additive tubes by gentle inversion 5 to 10 times immediately after the draw (Sodium citrate tube mix only 3-4 times). This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Prior to centrifugation serum separator tubes should be kept in vertical position until the clot formation. Centrifugation prior to clot formation can result in fibrin formation, which may interfere the test result.

Note: Patients on anticoagulant therapy may need longer time to clot

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z) Blood Sample Centrifugation

It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.
- All serum tubes for 15 minutes at 3500RPM and sodium citrate tube for 30 minutes at 2500RPM
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.

Limitations:

- Do not disturb the red button which are the red cells that have been separated.
- Label the secondary tube with proper patient identity before transferring the centrifuged samples (serum, Plasma ect.)
- Do not place unspun tubes in the refrigerator. (The refrigeration of the unspun tube will increase Potassium levels by 135 %.)
- Light Sensitive requirement: Based on the sample collection instruction for special tests pour plasma/serum into a dark aliquot tube to protect the specimen from any light source to ensure specimen integrity. If a dark aliquot tube is not available, wrap aluminum foil or paper towel around the tube (not the stopper)tightly. Please ensure the tube will not be exposed to light during storage and transport. The patient identification should be easily accessible to view by sliding the tube from the wrapping which is why the stopper end of the tube is not covered.

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6. General Centrifuge Safety

SCOPE: The following information is simply meant to provide guidance in assisting to develop centrifuge safety and to ensure that all centrifuges in laboratories are used, cared for and maintained in a safe manner.

RESPONSIBILITY: It is the responsibility of the all handling technician, lab Director, Q&T Manager and Deputy Q&T Manager

EMPLOYEY PROTECTION: Users should be wearing appropriate laboratory PPE

MATERIALS:

- Centrifugation mode :
 - ✓ When in normal mode, pressing START key starts the centrifugation.
 - ✓ STOP key can be used to abort the process and come back to normal mode
 - ✓ BRAKE function can be toggled with help of respective key.
 - ✓ Decimal point on the TIMER display blinks which indicates that the down counting is going on.
- Centrifuge loads must be balanced by placing the tubes of equal size, shape and containing equal volumes opposite to each other, do not over load the centrifuge

PROCEDURE: General Safety Measures

Centrifuges are instruments with strong potential for harming users due to the high speed at which they operate: mechanical failure of the rotor can result in injury, even death; and sample container breakage can generate aerosols that are harmful to inhale. Thus, it is very important to act safely when using and maintaining these instruments.

Handling Procedures: To avoid accidents and injury, always follow the manufacturer’s operating instructions for the centrifuge being used.

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Inspect centrifuge: Look over the centrifuge and rotor prior to use.

1. If visible, ensure centrifuge spindle is clean.
2. Check that rotor, safety cups and/or buckets do not have signs of corrosion, cracks or deformities.
3. Ensure centrifuge and rotor are dry.
4. Make sure the rotor is properly seated on the drive shaft.
5. Check that safety cups/rotors are properly seated and able to move freely. Check Swing out rotors are designed to be used with all buckets present, even if some of them are empty. Make sure bucket pairs (symmetrically balanced with the tubes) are the same type.

Prepare samples:

1. Select appropriate tubes or containers for rotor, sample and speed.
 - Tube/container and rotor bottoms must match. E.g. conical bottom rotors need conical bottom tubes.
 - Sample must be compatible with tube/container material.
 - Tube/container must be rated for speed being used.
2. Inspect tubes and containers for cracks or flaws before using.
3. Avoid overfilling or under filling tubes and containers, make sure to follow manufacturer limits when given.
5. Balance tubes, see the Balance rotor and containers
6. Make sure the exterior of the tubes and/or containers are clean and dry prior to centrifugation.

Run centrifuge

- Ensure lid of centrifuge is properly closed.

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- Set run speed and time, never exceed the rotor's maximum run speed.
- Do not leave the centrifuge until full operating speed is reached and the machine appears to be running safely.
- Stop the centrifuge immediately if you notice any unusual noises or shaking.
 - Confirm rotor is properly seated and balanced.
 - If problems persist, discontinue use and contact supervisor.
 - Do not use centrifuge until it has be serviced by a qualified technician.
- Make sure the rotor has come to a complete stop before opening the lid.
- When closing the centrifuge lid do not place your fingers between the lid of the centrifuge
- Glass tubes must be run within the speed limit according manufacture.

Procedure for Spills inside a Centrifuge

1. Check for leaks and spills after each run.
2. If you know, or suspect, a spill has occurred, Turn off the centrifuge immediately and do not open for 10 minutes to reduce aerosolization.
3. Put on personal protective equipment (gloves, lab coat, and face protection).
4. Cover the contaminated inner surfaces of the centrifuge with paper towels and soak with disinfectant.
5. Allow 10 minute disinfectant contact time.
6. Use forceps to remove all pieces of broken glass. Place broken glass inside biohazard sharps container.
7. Wipe down all surfaces inside the centrifuge a second time with disinfectant. Dispose of waste in biohazard container.
8. Remove gloves and other protective equipment.
9. Thoroughly wash hands with soap and water.

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Mechanical failure

- If the machine experienced problems during a run, turn off the centrifuge immediately and unplug the power cord. Post warning signs so no one plugs the machine back in.
- Do not use the machine until it has been inspected and repaired by a qualified service technician.

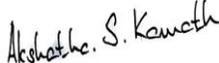
Preventive Maintenance: It is important to keep centrifuges running optimally. These are expensive pieces of equipment and the better they are maintained the longer they will last.

Establish a maintenance schedule:

- Consult the operator's manual or contact manufacturer for information regarding regular servicing.
- Clean the interior of the centrifuge and rotor(s) regularly to prevent damage.
- Only wash the buckets of a swinging bucket rotor. The body of the rotor should never be immersed to prevent rusting.
- Thoroughly rinse all washed pieces with water and then air dry
- Store rotors and buckets upside down in a dry environment, not inside the centrifuge.
- Follow manufacturer recommendations or contact the manufacturer for guidance.

Cleaning

- Daily cleaning inside the centrifuge with dry cloth
- To clean centrifuge tubes: Wash with mild detergent in warm water, rinse them thoroughly with clean water and allow them to air dry.
- Appropriate cleaning of reusable tubes is necessary to prolong their life and avoid having them break or collapse during centrifugation.

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- Spillage tubes only if absolutely necessary, tubes can be sterilized by clinical methods, dry heat or autoclaved up to 120°C.

Emergency Procedure

In the event that an incident or accident related to centrifugation occurs:

- Turn off centrifuge and disconnect it from the power source
- Notify others in laboratory and evacuate
- Notify the lab supervisor

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7. COLLECTION OF URINE SAMPLE

- **Purpose:** Collection and Handling of Urine Samples, Stool samples and Semen Samples
- **Scope:** to define the urine/Stool/Semen collection procedures for all sections including Biochemistry, Microbiology and Clinical Pathology
- **Responsibility:** Lab technician/Q&T Manager and Deputy Q&T Manager/laboratory Consultants/ Lab Director
- **Procedure:** as under

- **Specimen collection and handling**

Each request for a urine/stool/semen specimen / any type of sample collection should be accessioned to identify all paperwork and supplies associated with each patient.

- Accession is in the form of entry of integration into the system with raising request and barcode (in the Out Patient Sample Collection Register by the registration assistants and affixing a number on the request form.)
- Appropriate containers should be labeled barcoded and given to the patients with proper instructions for collection and handling of specimens.

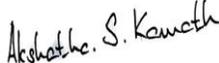
- **Approaches and Identify the Patient**

1. Out Patient Sample Collection

- The technician should identify himself or herself, explain the procedure to the patient and gain the patient's confidence.

2. In Patient Sample Collection

- The doctor / paramedical staff / nurse/technicians responsible for collection of sample. He/she shall identify himself or herself, establish a rapport, and gain the patient's confidence.

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3. Identify Patient

- Identification of the patient is crucial. The technician must ensure that the urine/stool/semen specimen is belongs to the individual designated on the request form. The following steps are a suggested for ensuring patient identification regardless of the clinical setting.

➤ **Patient who is Conscious**

The steps are as follows:

- Ask the patients to give out full name, address and identification number (Hospital number).
- Compare this information with the information on the request form.
- In case of any discrepancy, report to the senior / In charge / doctor for clarification.

➤ **Patient who is Semiconscious, Comatose or Sleeping (In patient)**

The steps are as follows:

- Ask the relative/kin/nursing staff to give out full name, address and identification number (Hospital number).
- Compare this information with the information on the request form.
- In case of any discrepancy, report to the senior / In charge / doctor for clarification.

Patient who is Unconscious, Too Young, Mentally Incompetent, or does not speak the Language of the Person Responsible for Sample Collection *

In any of these circumstances, the following steps are suggested:

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- Ask the relative or a friend to identify the patient by name, address and hospital number.
- Compare the data with the information on the patient's chart or the request form.
- In case of any discrepancy, report to the senior / In charge / doctor for clarification.

Procedure for Identifying Unidentified Emergency Patients *

- The patient must be positively identified when the specimen is collected. The unidentified emergency patient should be given temporary but clear designation until positive identification can be made.

➤ **Verify patient Diet Restrictions/Abstinence**

Some tests require the patient to eliminate certain foods from the diet before the urine/stool/semen sample is collected. Time and diet restrictions vary according to the test. Such restrictions are necessary to ensure accurate test results. Proper abstinence as per the annexure should be followed before semen sample collection (**See Annexure**)

➤ **Label Urine/stool/semen Container and Record Time of Collection**

The patient and the patient's urine/stool/semen specimen must be positively identified at the time of collection. Specimens must be obtained in the appropriate container tubes identified with a label bearing at least the following.

- The patient's first and last name
- Age/sex
- An identification number (Hospital MR number)
- The date

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- The time
The completed label must be attached to the sample container tube before leaving the side of the patient. The laboratory documents the time when the specimen was collected. Whenever possible, a small signature or initials of the personnel responsible for collecting the specimen shall be recorded.

Urine specimen collection and handling of urine testing

Types of collection: Laboratory urine specimens are classified by the type of collection conducted or by the collection procedure used to obtain the specimen.

Random Specimen

- Most commonly sent to the laboratory for analysis
- it is the easiest to obtain
- As the name implies, the random specimen can be collected at any time of the day.
- Clean-Catch Prior to collection, clean the external genitalia with a mild antiseptic solution, wash with water and dry. Void off Allow the initial portion of the urine stream (to escape,) and collect the midstream portion in a sterile container. (And allow the final portion to escape). If a urine culture is not ordered, it is not necessary to void into a sterile container.
- A clean dry container may be used for urinalysis.

First Morning Specimen

- This is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated and, therefore, contains relatively higher levels of cellular elements and analytes.
- The first morning specimen is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep.

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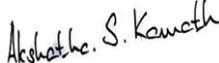


24 hour or Timed Collection Specimen

- For measuring protein, creatinine, urine urea nitrogen, glucose, sodium, potassium, or analytes such as catecholamines and 17-hydroxysteroids that are affected by diurnal variations.
- A timed specimen is collected to measure the concentration of these substances in urine over a specified length of time, usually 24 hours.
- In this method, after getting up in the morning the first urine is discarded. All urine voided subsequently during the rest of the day and night is collected in a large bottle 2liter capacity with a cap. The first urine after getting up in the morning on the next day is also collected. The urine should be pursed at 4 – 6oc during the period of collection. The container is then immediately transported to the laboratory. In case of any delay 10ml of 6N HCL can be used as a preservative.
- In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. When the 24-hour urine output is 2 litre, 10 mL of 6N HCl can be used as a preservative.
- **Test names - preservative**
 - Copper and Arsenic – 6N HCL,
 - Catecholamines PO₄ – 25ml of 50% acetic acid
 - Cortisol -10gm boric acid
 - Calcium – 25ml of 50% conc.HCL AND
 - Aldosterone – 1gm boric acid/100ml urine

Urine collection containers

- Urine Collection Containers -leak-resistant cups/bottles (sterile and non-sterile)
- Sterile for **urine culture test** and Non sterile bottles for **Urine analysis**

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- Urine Collection Containers (24-hour collection) Urine collection containers for 24-hour specimens are of 3-4 liter (L) capacity and are amber colored (to protect light-sensitive analytes such as porphyrins and urobilinogen). When a preservative is required, it should be added to the collection container before the urine collection begins.

Preservatives for Urinalysis testing urine within 1-2 hours of voiding (two hours of its collection) is recommended. However, refrigeration or chemical preservation of urine specimens may be utilized if testing or refrigeration within a two-hour window is exceeded (not possible). A urine preservative of 6N HCl is available that allow urine to be kept at room temperature.

Specimen collection and transport guidelines

24 hr urine collection

- Instruct the patient that the preservative in container is acid and should be handled with caution.
- Collect each void in smaller clean container and pour the urine into the 24 hr urine container. Do not pass urine directly into 24 hr urine container.

Random urine collection

- All urine collection and/or transport containers should be clean and free of particles or interfering substances.
- The collection and/or transport container should have a secure lid and be leak-resistant.
- Use containers that are made of break-resistant plastic, which is safer than glass.

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- Specimen containers should not be reused.
- Catheterized specimens should not be routinely obtained for urinalysis although they may be submitted

Urine specimen handling guidelines

Labels

- Include the patient name and identification on labels.
- Make sure that the information on the container label and the requisition match.
- If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed on a different container.
- Ensure that the labels used on the containers are adherent under refrigerated conditions.

Volume

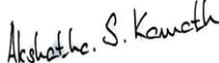
- Ensure that there is sufficient volume to fill the tubes and/or perform the tests.
- Under filling or overfilling containers with preservatives may affect specimen-to-additive ratios for chemical analysis.

Collection Date and Time

- Include collection time and date on the specimen label. This will confirm that the collection was done correctly. For timed specimens, verify start and stop times of collection.

Collection Method

- The method of collection should be checked when the specimen is received in the laboratory to ensure the type of specimen submitted meets the needs of the test ordered.

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An example of an optimum specimen/test match would be a first morning specimen for urinalysis and microscopic examination.

Proper Preservation

- Check if there is a chemical preservative present or if the specimen has not been refrigerated for greater than two hours post collection. After accepting the test request, ensure that the method of preservation used is appropriate for the selected test.

Light Protection

- Verify that specimens submitted for testing of light-sensitive analytes are collected in containers that protect the specimen from light.

Specimens stored at room temperature should be delivered to the laboratory within 2 hours after collection. Refrigerated specimens are accepted for up to 24 hours after collection.

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8. COLLECTION OF STOOL SAMPLE

Specimen Collection

➤ **Do's**

- A wide mouthed jar with a screw cap is good, provided it is neat, clean and without any extraneous material in it.
- Should be opened slowly to release the gas that accumulates frequently in it.
- Since rectal evacuation is not completely at will and faeces passed correlate very poorly with the food consumed, hence collection should be done over a period of three days.
- Stool should not be contaminated with urine, water and menstrual bleed. (Faeces should be urine free when collected.) Urine should be passed before the stool collection
- Collect the entire stool and transfer to another container by a tongue blade. About 20-40gm of formed stool or 5-6 table spoon of watery stool should be collected Only a small amount of stool is needed, roughly the size of a walnut
- If mucus and blood are present, they should be included in part of the specimen to be examined
- Deliver to the laboratory immediately after collection(preferably within an hour for receipt) a fixative containing 10% formalin may be used if specimen is to be transported to a distant laboratory
- Refrigerate stool only if it cannot be examined immediately.
- A diarrhoeal stool usually gives good results
- Preferably stool specimen should be collected before antibiotic therapy is initiated and as early in the course of the disease as possible

➤ **Dont's**

- Do not use a stool that has been passed into the toilet bowl or that has been contaminated with barium or other X ray medium

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- It should never be overfilled
 - Stool specimen should not be mixed with toilet paper, water or soap
 - Do not refrigerate for ova and parasites
 - Stool specimen should never be kept in an incubator
- **Fetal Occult Blood Test**
- The fecal occult blood test requires the collection of three stool samples. The stool samples should be taken one day apart, because colon cancers may bleed from time to time, rather than consistently.
 - The fecal occult blood test results are largely affected by how the patient prepares for the test, so it is important to follow the instructions carefully.
- **Dont's.**
- Do not perform the test if the patient has: Diarrhea, Colitis, Constipation, Diverticulitis, Ulcers, Hemorrhoid flare-ups, and Menstrual period
- **The following foods should not be eaten 48 to 72 hours before taking the test:**
- Beets, Broccoli, Cantaloupe, Carrots, Cauliflower, Cucumbers, Grapefruit, Horseradish, Mushrooms, Radishes, Red meat (especially meat that is cooked rare), Turnips, Vitamin C-enriched foods or beverages.
 - As for all occult blood tests certain medication such as aspirin, indomethacin, Phenylbutazone, reserpine, corticosteroids and nonsteroidal anti-inflammatory drug can induce gastrointestinal bleeding and cause false positive results. These medications should be temporarily discontinued with the consent of the physician for 7 days prior to testing and during the test period.

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➤ **Collection of Stool for Ova & Parasite**

- a. Antacids, barium, bismuth, anti-diarrhea medication or oily laxatives should not be used prior to collection of the specimen.
- b. For optimal recovery of all parasites, samples should be submitted to the laboratory, unpreserved and in accordance with the following time frame:

Liquid Stool: Must be received within 30 minutes of collection

Soft Stool: Must be received within 30 minutes of collection

Semi-soft Stool: Must be received with in 1 hour of collection

Formed Stool: Must be received on the same day of collection

- Ask the patient to pass the stool sample directly into a sterile screw capped container
- About 20-40 grams of well-formed stool or 5-6 tablespoonful's of watery stool for a routine examination.
- Ingestion of some medicines prior to collection of faecal sample may interfere with the detection of parasites. These include tetracyclines, sulfonamides, antiprotozoal agents, laxatives, antacids, castor oil, magnesium hydroxide, barium sulphate, bismuth kaolin compounds and hyper tonic salts etc. These should not be taken 1-2 weeks before the examination of stool sample.
- Stool specimens should never be frozen and thawed or placed in an incubator because parasitic forms deteriorate very rapidly.
- Instruct the patient to collect the sample in clean container

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- **Send Sample Containers to Laboratory**
Appropriately labeled urine/stool/semen containers should be sent to laboratory designated to perform the required testing procedures.
- **Transportation of primary sample with specification about time frame, temperature and carrier**

From IPD wards / floors to Laboratory Reception

- The samples are collected by the TRAINED PERSONNEL
- Samples are transported through lab assistant and documented in sample dispatch register.
- The specimens are checked that the proper identification of the patient (name, IPD no.) and the tests to be performed are marked.
- It is verified that the specimen are OK for testing as per SOP Rejection of primary sample
- Once the specimen is OK it is allotted a unique identification lab number and the steps as per A above are followed.

➤ **From OPD Sample Collection room to Laboratory Reception**

- The samples are collected and properly marked with the patient name and its unique identification lab number.
- TO CHECK THE slip is attached for the tests to be performed on each specimen with the time of draw.
- All specimens are kept in the box / tray and transported to lab within 30 minutes.
- The specimen that needs to be processed immediately and for urgent report, they are sent to lab as soon as they are collected.

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➤ **Rejection of primary samples as per the respective SCM**

➤ **Receipt, labeling, processing and reporting of primary sample**

To receive a complete requisition form W.R.T

- Name of the physician or other person legally authorized to make request for examination
- Type of primary sample and the anatomic site of origin.
- Examination requested.
- Clinical information of the patient for interpretation purpose
- Date and time of receipt of sample in the laboratory

Unique identification of the patient **TO BE GIVEN BY LAB PERSONNEL** as per the individual sections (every hospital needs to define it as per their requirement)

➤ **Receipt, labeling, processing and reporting of primary sample in case of urgent sample request**

Criteria For Urgency/Emergency Must Be Defined At Individual Hospital Level

- The samples which are marked with word “Urgent” on patient requisition forms are sent to the lab reception on urgent basis from both wards and OPD collection room by ward boys.
- This information for urgent samples may also be communicated telephonically to the reception and respective sections of lab.
- The specimens are checked for the proper identification as per SCM Rejection of primary samples

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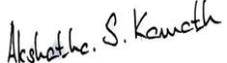
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- Once the specimen is found OK it is allotted a unique identification lab number
- Then the samples are specially transported to the respective sections of the lab on priority basis by available ward boys or reception staff

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9. MICROBIOLOGY DEPARTMENT COLLECTION OF SAMPLES

Purpose: To conduct cultures of various samples including Blood culture, Cerebrospinal Fluid culture, pus, body fluids, urine, sputum, stool and others. To determine the bacterial growth and perform antimicrobial susceptibility testing for diagnostic and therapeutic purpose

Scope: To define the collection procedures for Microbiology and Infectious disease serology.

Principles of collection:

1. Specimen should be collected under strict aseptic conditions.
2. It is necessary to avoid contaminating discharges. (Or ulcer material) with skin commensals.
3. Specimens should be collected in dry sterile, leak proof containers free from all traces of disinfectant.
4. Each specimen must be clearly labeled with a) Patient's name b) date c) time and d) Ward (if necessary)
5. Each specimen should be accompanied by a request from which gives:
 - a. Patient's above mentioned data
 - b. Investigation required and
 - c. Clinical note giving details of
 - the patients
 - suspected diagnosis and
 - any antimicrobial treatment (that may have started)
6. Collect the specimen at optimal times (Early morning sputum and urine specimen, whenever possible collect specimens prior to administration of antimicrobials)
7. Specimen should be sent in tightly sealed containers with no external spillage.

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8. All specimens (especially CSF, Blood, Wound specimens, specimens suspected to contain anaerobes) should be promptly delivered to laboratory after the collection to ensure minimum delay and prompt processing.

Responsibility: Lab Incharge/Microbiologist

Storage of the Collected Material:-

Refrigeration at 2-8°C can help to preserve cells and reduce the multiplication of commensals. The fastidious organisms such as *S. pneumoniae* and *H. influenzae* require immediate culturing. Hence the specimen should be inoculated as early as possible.

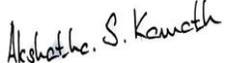
Specimens that should be refrigerated:-

1. Urine
2. Sputum
3. Faeces /Stool.
4. Pus/ wound specimens.
5. Respiratory specimens.
6. All catheter tips.

Specimens that should not be refrigerated:-

1. Blood.
2. Body fluids for culture.

Criteria for rejection of specimens:-

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- Unlabeled or incorrectly labeled specimens
- Specimens received without a request form:
- Request forms received without specimen:
- Specimens received with a request form devoid of any patient demographic details
- Missing vital information in the requisition form
- Specimen received in fixative (formalin)
- Specimens are received in improper or non-sterile containers, leaking containers, or transport media
- Specimens received that have been delayed in transit
- The specimen has been transported at the improper temperature
- Dry swab:
- Foley catheter tip:
- Unpreserved urine held in the refrigerator for >24 hours:
- Sputum specimen with 25 < WBC, >10 epithelial cells/lpf:
- 24-h collection of urine.
- Gram stain for *Neisseria gonorrhoeae* on specimens from cervix, vagina, and anal crypts:.
- Inadequate specimens-If only one swab is submitted with multiple requests for various organisms (bacteria, AFB, fungi, virus, ureaplasma, etc.), ask the physician to send the additional samples or to prioritize test requests.

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- Excess specimens-If more than one specimen of urine, stool, sputum, wound or routine throat specimens were submitted on the same day from the same source. Notify the physician or charge nurse that, only one specimen will be processed per day.

**Method Of Collection And Transportation:-
Blood Culture and Sensitivity**

First cleanse the vein puncture site with chlorhexidine-alcohol solution to disinfect the site using progressively larger concentric circles. Disinfectant should remain in contact with skin for about 1 minute or until dry to ensure disinfection. The vein puncture site must not be palpated after preparation. Blood is then drawn.

Blood cultures should be drawn prior to initiation of antimicrobial therapy.

If more than one culture is ordered, the specimens should be drawn separately at no less than 30 minutes apart to rule out the possibility of transient bacteremia by self-manipulation by the patient of mucous membranes in the mouth caused by brushing teeth, etc or by local irritations caused by scratching of the skin.

Strict aseptic technique is essential.

If present remove the plastic cap from the blood culture bottles, swab the stoppers with 70% alcohol. And allow to dry.

Collect 10 mL blood in a sterile plastic syringe and inoculate at least 10 mL blood (as indicated on bottle) into each bottle.

Urine culture and Sensitivity

Clean – voided midstream urine is to be collected in a sterile container supplied by hospital.

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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 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 rinsed 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) for urine culture

- Catheter is clamped above the distal end to collect freshly voided urine.
- Tubing is cleaned vigorously with 70% alcohol (spirit).
- Under all aseptic precautions, urine is aspirated with needle & syringe. Then, urine is collected in wide mouth sterile container. Never collect urine from Uro-bag.

Midstream urine is collected by the patient. If disabled, nursing staff will assist in collection.

For catheterized specimen, nursing staff will collect the specimen. Suprapubic aspiration is performed by the physician.

Sample volume -10 ml

Transportation time : Within 2 hrs at room temperature , if delay anticipated to be kept in refrigerator at 2-8 degree Celsius

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Conjunctival /Eye specimens:-

Eye specimens should be collected by a medical specialist, an experienced technologist or nurse.

1. Collect discharge or swab each eye with separate swabs by rolling over conjunctiva.
2. Corneal scrapings – Collected by ophthalmologist. Send in sterile container or preferably inoculate directly onto media.
3. Vitreous fluid – Prepare eye for needle aspiration of fluid. Transfer fluid to sterile tube.
4. Swabs can be transported in BHI transport medium. Transport at the earliest.

Ear specimens

Ear specimens should be collected by a medical specialist, an experienced technologist or nurse.

1. Aspirate discharge and collect in a sterile container or collect with a sterile swab. Transport at the earliest. Transport medium can be used for transportation.

Sample is collected by the treating doctor.

Vaginal Swab:-

1. Collect vaginal secretions from the mucosa high in vagina and transfer to sterile container or discharge on a sterile cotton swab stick.

Two swabs should be collected one for gram stain and another for culture

Sample is collected by the treating doctor.

Specimen should be processed within 2 hours, if processing delayed should be kept in refrigerator at (2-8⁰C).

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Body Fluids, Sterile (Except Urine & CSF): -

It must be collected by an experienced medical officer. The collection is performed under strict aseptic conditions in sterile container and transported to the laboratory immediately at ambient temperature.

Submit 10 mL of the specimen for analysis.

CSF Culture: -

It must be collected by an experienced medical officer. The collection is performed under strict aseptic conditions in sterile container.

Collect an adequate volume of fluid as recommended below.

- ⇒ Bacterial culture > 1 mL
- ⇒ Fungal culture 5-10 mL
- ⇒ Mycobacterial culture 5-10 mL

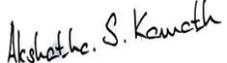
Transport the specimen at ambient temperature. If a delay in transport occurs, incubate at 37°C or leave the fluid at ambient temperature for transport.

Pus/ Wounds Culture: -

Examination of pus from Wound abscesses, burns and sinuses.

Closed wound:

- Cleanse the skin as for blood cultures.
- Aspirate the fluid/purulent material using a sterile needle and syringe and transfer to sterile container.

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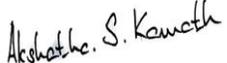
- If no material is obtained, unroof vesicle or bullous lesion and use a swab to collect cells from the base of the lesion. Place in BHI / sterile saline/ transport medium media and send to laboratory.
- Two swabs are generally collected. One is used for direct microscopic examination and the other is used for the culture.

Open wound: Specimens are collected from wounds (ulceration) of different part of the body by touching the infected area with a sterile swab. The swab should be placed immediately in BHI / sterile saline/ transport medium and send to laboratory.

- Clean the sides of the wound surface mechanically, without using a germicidal agent, to remove as much of the superficial flora as possible.
- Collect the pus of fluid if possible in a syringe, or collect the discharge in a sterile cotton swab.
- Place in appropriate bacterial transport media, if delay is expected.
- Two swabs are generally collected. One is used for direct microscopic examination and the other is used for the culture.

Note:

If the infection is suspected to be due to an anaerobe, aspirate the draining pus into a sterile syringe and immediately put it into a thioglycollate broth.

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Sputum Culture:

- Early morning sputum is the preferred sputum specimen.
- Sterile, wide mouth container should be used for specimen collection.
- The patient is instructed to rinse his/her mouth with plain water before bringing up the sputum.
- Instruct the patient to inhale deeply 2-3 times, cough up deeply from the chest and spit in the sputum container by bringing it closer to mouth.
- Make sure the sputum specimen is of good quality and not saliva. A good sputum specimen is thick, purulent and sufficient in amount (2-3 ml).
- Transport immediately at ambient temperature. Refrigerate (2-8°C) if a delay of more than one hour is anticipated
- Assure patient cooperation to get an adequate specimen.
(Salivary samples will be rejected as per the Laboratory Rejection Criteria.)

Semen Culture:

- Sexual abstinence is required for atleast 3days and not more than 7 days.
- Instruct the patient to pass urine before specimen collection.
- The patient is advised to wash hands and penis with soap and water .Dry thoroughly before collecting the specimen.
- In a sterile container semen is to be collected by masturbation.
- Lubricants, condoms should not be used for specimen collection.
- If transported, to be transported at the earliest at ambient temperature. Do not expose to extremes of temperature.
- Semen should be accepted only for walk-in patients within half-hour of collection.

Bronchial Brush/Washing/Lavage/Aspirate:-

This technique should be performed by an experienced medical officer, Nurse.

Collect in sterile leak proof container. Transport in a sterile container at 2-8°C for cultures.

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Urethral Discharge:-

1. Do not allow patient to urinate for at least one hour prior to collection.
2. Take a sterile swab moistened with sterile normal saline.
3. Collect a specimen of pus/purulent discharge on a sterile cotton swab. Insert the swab in BHI transport medium by maintaining aseptic conditions as far as possible. The specimen is transported immediately at ambient temperature.
4. For Gram staining make a smear of the discharge on a slide.

Genital specimens

Males Collect the specimen in the morning before the patient has voided urine.

- If necessary, clean the meatus with a swab moistened in normal saline or plain lukewarm water.
- Exert a slight pressure on penis so that a drop of pus appears at meatus.
- Remove the pus with a sterile inoculating loop or apply directly to a clean glass slide.
- If no pus appears, obtain by prostatic massage.
- Prepare smears on two different slides by spreading the smear as thinly as possible.
- Anorectal specimens are collected by inserting a swab 4-5 cm into the anal canal.

Females

- Discharge/swab is collected by a medical officer/gynaecologist.
- In case of chronic gonorrhoea, the specimen should be taken just before or after the menstrual period.
- Insert the sterile swab 2-3 cm into the cervical canal and rotate it for 5-10 seconds to permit absorption of the exudate.
- Before inoculating the culture or transport medium, it is desirable to prepare a smear for microscopy. Prepare the slides in duplicate by rubbing the pus swabs over a clean slide in a thin film. To obtain a thin homogenous film, roll swab onto a clean slide and allow the smear to air-dry.

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For Neisseria gonorrhoeae, the material is directly inoculated onto culture media.

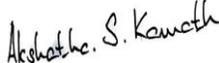
Throat Swab Culture:-

Swab should be collected by a medical officer or by a experienced technician.

- Use a cotton or Dacron swab.
- Use a tongue blade and an adequate light source to ensure proper visualization.
- Examine the inside of mouth. Look for inflammation, exudates, pus or presence of any membrane.
- Depress the tongue.
- Swab the inflamed area of the throat, pharynx or tonsils with a sterile swab taking care to collect the pus or piece of membrane.
- Take care not to touch the buccal mucosa or tongue (prevent contamination with saliva).
- Sterile swab in a tube with a cap. (Two swabs, one for gram stain, another one for culture).
- Transport in sterile transport tube.

Note the following:

Disease	Observation
Diphtheria	Grayish-yellow membrane extending over the soft palate and backwards onto the pharyngeal wall.
Streptococcal sore throat	Tonsils are covered with yellow spot and are inflamed.
Infectious mononucleosis	Exudates.

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- Send the collected swab immediately to the laboratory or put in BHI / sterile saline/ transport medium if delay expected.

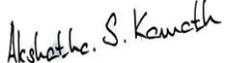
Nasal Swab Culture:-

- This is an inappropriate specimen for anything other than assessment of staphylococcal colonization (MRSA screening).
- Insert the sterile cotton swab gently into one or both anterior nares rotate to collect mucus membrane cells and withdraw.
- Send the collected swab immediately to the laboratory or put in BHI / sterile saline if delay expected.

Stool Culture:-

Faecal specimens for the etiological diagnosis of acute infectious diarrhea should be collected in the early stage of illness and prior to treatment with antimicrobials. A stool specimen rather than a rectal swab is preferred.

- Antacids, anti-diarrhea medication or oily laxatives should not be used prior to collection of the specimen.
- Do not collect more than 2 specimens/patient without prior consultation with clinician.
- The faeces specimen should not be contaminated with urine.
- Do not collect the specimen from bed pan.
- Collect the specimen during the early phase of the disease and as far as possible before the administration of antimicrobial agents.
- 1 to 2 gm quantity is sufficient.
- Place specimen in a clean leak proof wide mouth container and transport to lab within 2 hours. If more than 2-hour delay is expected then transport under refrigeration (2-8°C).
- If a stool specimen is not available, the following are suitable alternatives for culture:
 1. A swab of rectal mucus, or

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2. A rectal swab inserted one inch into the anal canal .The swabs should be inoculated immediately onto culture media.

Tissue for Culture:-

Sterile instruments should be used for each tissue. Place each tissue into separate sterile container with sterile saline and transport the specimen at ambient temperature.

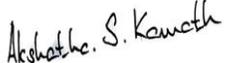
Acid Fast Bacilli Culture:-

Respiratory secretions [sputum, bronchial washing, bronchoalveolar lavage, bronchial brushings], urine, stool, CSF, body fluids, whole blood and tissue biopsies.

- a. Collect in a sterile leak proof container.
- b. Collect a minimum of 3-5 early morning sputum.
- c. For urine AFB earl morning urine sample (3-5 ml)
- c. If body fluids are collected, collect 5-10 mL body fluids.
- d. Send all specimens to main lab immediately.
- e. Respiratory, urine and stool specimens should be sent in cold pack.
- f. Whole blood, tissue and CSF specimens should be sent at ambient temperature.

Ophthalmological sample for Culture:-

- Conjunctivitis –Swab of bilateral conjunctiva is inoculated in culture media
- Keratitis – Scraping of affected lesion and material is inoculated directly onto appropriate media by the ophthalmologist.

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Anaerobic culture

- Swabs are not the preferred collection method for anaerobic culture.
- Draining pus or aspirate is the preferred specimen for anaerobic culture.
- Specimens likely to contain anaerobes as normal flora are not ideal for anaerobic culture. These include coughed sputum, gastric contents, skin/ulcers/decubitus/superficial wounds, feces for C.difficile, urine, nasopharynx secretions, vaginal secretions, cervical, uterine, seminal fluid, perirectal, BALs, and small bowel contents.
- **Cutaneous (fungus only):-**
 1. **Hair:**
 - Scrape the scalp with a blunt scalpel.
 - Place specimen in a dry sterile container.
 - Transport at ambient temperature.
 - The following specimens are also acceptable:
 - Hair stubs
 - Contents of plugged follicles
 - Skin scales
 - Hair plucked from the scalp with forceps

Note: Cut hair is NOT an acceptable specimen.

2. Nails:

- Cleanse the nail with 70-95% ALC.
- Remove the outermost layer by scraping with a scalpel.
- Place specimen in a dry, sterile container.
- Transport at ambient temperature.
- The following specimens are also acceptable:
 - Clippings from any discolored or brittle parts of nail.
 - Deeper scrapings and debris under the edges of the nail.

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Prepared By:	 Ms Anasooya Quality & Technical Manager	Approved By:	 Ms Anasooya Quality & Technical Manager	Issued By:	 Dr Akshatha Kamath Lab Director
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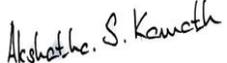
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3. Skin:

5. Cleanse the skin with 70-95% alcohol.
6. Collect epidermal scales with a scalpel, at the active border of the lesion.
7. Place specimen in a dry, sterile container.
8. Transport at ambient temperature.

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