

Vardhman Mahavir Medical College & Safdarjung Hospital	Clinical Biochemistry Unit, Department of Biochemistry	
	Document Name	Sample Collection Manual
	Document Number	SJH/CBU/BIOCHEM LAB/Manuals/SCM/03

RELEASE AUTHORISATION

SAMPLE COLLECTION MANUAL

Of

Clinical Biochemistry Unit & NEB Biochemistry Lab

Safdarjung Hospital, Delhi

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Issue no: 02

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Amendment: 01

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Total pages , sections and annexures of safety manual, checked verified and approved

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Prepared by: Dr Deepa Halder		Reviewed by: Dr Sukanya Gangopadhyay Dr Anita Rani		Approved by: Dr Anita Rani	Page 1 of 26

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1	Page no. 2-3	02	01	12.08.2022	Amendment page added		
2	Page no. 4-5	02	01	12.08.2022	Index added		
3	Page no. 10-11	02	01	12.08.2022	Preparation of patients for OGTT and GCT mentioned in detail		
4	Page no 26	02	01	12.08.2022	Criteria for rejection of samples modified as per policy of lab		

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1. Purpose: This document provides standardized approach for proper specimen collection.

2. Scope: To ensure proper specimen collection, labelling and handling of specimens.

3. Responsibility: Lab Director/QM/TM/Senior Residents and all technical staffs.

4. Procedure:

4.1. Blood sample collection and handling:

4.1.1. Every request for a blood specimen or any type of sample is accessioned to identify the patient from test requisition form (TRF).

4.1.2. Accession is in the form of entry in the patient sample collection register by the technician and affixing a lab number on the request form.

4.1.3. For blood collection, patients are then directed to sample collection counters according to the accession numbers which is displayed.

4.2. Sample collection centers

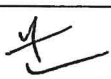
4.2.1. Central collection Centre:

4.2.1.1. Located in in Room No. 19 of OPD Block, VMMC & Safdarjung Hospital.

4.2.1.2. Here all OPD samples are collected from 8 AM to 11.30AM.

4.2.2. Clinical Biochemistry Unit:

4.2.2.1 Located in Room No. 415 of Clinical Biochemistry Unit, Old Casualty Block, VMMC &

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Safdarjung Hospital.

4.2.2.2. Prior appointments are given to OPD patients for tests like Blood Glucose(Fasting and PP) and Lipid Profile and then samples are collected on that particular day from 8:30AM to 11.30 AM.

4.2.2.3. All ward samples are received at the sample receiving counter from 09:00 AM to 11:30 AM.

4.2.3. Super Specialty Block:

4.2.3.1. Samples of super specialty block are collected in room no. 19, central collection center from 9-11.30AM

4.2.3.2. All SSB OPD samples are collected from 9:00 AM to 11.30AM.

4.2.4. New Emergency Block:

4.2.4.1. Located in first floor, New Emergency Block.

4.2.4.2. Here sample collection is not done, but samples are received round the clock from Triage, ICU and casualty wards.

4.2.4.3. Samples from other wards and SSB wards are received from 11:30 AM onwards till 9:00 AM next day.

5. Approaches to identify the patient:

Responsibility: Lab Technicians

5.1. Out-Patient Department (OPD)

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The phlebotomist first asks the patient's full name and residential address. This information should match with the information provided in the requisition form. In case of any discrepancy, it should be reported to senior resident or doctor on duty. Sampling should not be done against the patient's or guardian's verbal consent.

5.2. In-Patient Department (IPD)

5.2.1. Conscious Patient

5.2.1.1. The phlebotomist or the doctor on duty should ask the patient's full name, MRD number, DOB and residential address.

5.2.1.2. The above information should match with the information provided in the requisition form.

5.2.1.3. In case of any discrepancy, report to Senior Resident or Doctor on Duty.

5.2.2. Semiconscious Patient, Sleeping Patient

5.2.2.1. Sample should always be taken in awakened state. Sleeping patient must be awakened before taking the sample.

5.2.2.2. The phlebotomist or the doctor on duty should identify the patient correctly and check patient's full name, MRD number on the requisition form.

5.2.2.3. The above information should conform with the information in the case sheet or file of the patient. In case of any discrepancy report to Senior Resident or Doctor

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on Duty.

5.2.2.4. In such patients, the phlebotomist or the doctor on duty must anticipate any unexpected movement or jerk either while introducing the needle into the patient's arm or while it is in place in the arm. In the event of accidental removal of needle, the tourniquet should be quickly released and gauze pad should be readily available for application. In case of any accident like needle going deeper in to the arm, inform Senior Resident.

5.2.3. Unconscious Patient, Children, Mentally Incompetent Patient.

5.2.3.1. Venipuncture in unconscious patients:

Staff who is competent and trained in venipuncture will do the procedure in unconscious patients after verbal consent and verification of patient details from the patient attendant or accompanying police officer in case of MLC. The procedure should be fully documented. Ideally the blood sample should be taken from a vein which has no existing intravenous line usually the antecubital fossa. It is acceptable to take blood from a cannula, central or arterial line if no other site is available. The site used should be documented along with mention whether blood is venous or arterial. After withdrawal of blood sample, the site should be pressed with gauze piece.

5.2.3.2. Venipuncture in children:

Children are likely to make unexpected movements and assistance in holding them still is often required. Either a syringe or evacuated blood tube system may be used to collect specimen. A

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syringe should be either the tuberculin type or a 3ml capacity syringe except when a large volume of blood is required for analysis. A 21-23 gauge needle or 20-23 gauge butterfly needle with attached tubing is appropriate to collect specimens.

5.2.3.3. Venipuncture in mentally incompetent patients:

Check the identification of the patient by name, address and hospital number with the help of the patient's attendant. Confirm the information on the patient's case sheet. In case of any discrepancy, report to the Senior Resident or Doctor on Duty.

6. Diet Restrictions:

6.1. Responsibility: Laboratory Technical staff

6.2. For Fasting Blood glucose, fasting period is advised to be 8-10 hrs.

6.3. Glucose Challenge Test

It is Performed between 24 and 28 wks of gestation on all average and high risk pregnant patients. 50 gm of oral glucose load is given without regard to time of delay or time of last meal and measure venous plasma glucose after 1 hr.

If glucose \geq 140 mg/dl, then perform OGTT.

6.4 For OGTT

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Patient is advised 3 days of unrestricted diet (which contains at least 150gm of carbohydrate per day. The personnel responsible for collection of samples must ask whether the patient is fasting or not. Fasting means nothing by mouth not even black tea. For medications that influence blood glucose, instructions given by treating clinicians to be asked and followed. 10-16 hrs of fasting is required. This test should not be performed on hospital, acutely ill or inactive patient. The test should begin between 7 to 9 am.

Procedure: Measure fasting venous plasma glucose. Give a dose of 75 gm of glucose dissolved in 300 ml of water and ingested over 5 minutes.

At least 2 values must meet or exceed the following

Fasting -95 mg/dl

1 hour-180 mg/dl

2 hr-155 mg/dl

6.5. For Lipid profile, fasting period must be 12 to 14 hrs.

7. Assemble supplies:

7.1. Responsibility: Technicians

7.2. All the below mentioned supplies should be present where venipuncture is performed.

7.3. Needle, Needle holder, Vacutainers, syringe, prepackaged swab (70 % isopropyl alcohol), gloves, tourniquet, adhesive plaster, needle destroyer, sharps container, waste bin as per BMWM guidelines 2016, (Amendment rules 2018,2019).

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8. Patient position:

8.1. Patient in sitting position:

8.1.1. Patient's sample is NEVER taken in standing position.

8.1.2. Patient has to be seated in chair with arms to provide support and preventing falls.

8.1.3. The arm intended for venipuncture should be extended at the elbow to form a straight line from the shoulder to the wrist so that forearm is lower to the elbow.

8.2. Patient in lying position:

8.2.1. Patient to be made to lie supine.

8.2.2. The arm intended for venipuncture should be extended at the elbow to form a straight line from the shoulder to the wrist.

8.2.3. A pillow under the arm intended for venipuncture can be kept for additional support.

9. Tourniquet:

9.1. **Location:** Wrap the tourniquet 4 to 6 inches above the venipuncture site.

9.2. **Material:** A pre-cut soft rubber strip or Velcro is used.

9.3. If blood pressure cuff is used as a tourniquet, inflate it to 60 mmHg.

9.4. A tourniquet should be applied over the patient's gown or a piece of gauze pad or tissue paper, in case the patient has some skin problem.

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9.5. It is advised to fasten tight enough to restrict venous flow but not arterial flow. This distends the veins making them larger and easier to find and stretches the walls so they are thinner and easier to pierce.

9.6. Factors to avoid

9.6.1. Never put tourniquet for more than one minute.

9.6.2. Pumping of fist before venipuncture to be avoided.

10. Vein selection:

10.1. The median cubital vein in the ante cubital fossa of upper limb is the first choice.

10.2. Cephalic vein in the ante cubital fossa of upper limb is the second choice.

10.3. Vein on the back of hand and at ankle can also be used.

10.4. Veins on the underside of the wrist should never be used.

10.5. In ward patients, sample from the cannula at the time of first insertion of needle should be taken to avoid second prick.

10.6. Procedure for selection of vein

Palpate and trace the path of vein with the index finger. A tourniquet must be used as an aid to the selection of vein unless specific tests do not require tourniquet. Patient should be asked to form a fist to make veins prominent.

10.7. Factors to avoid

10.7.1. Arm containing cannula and AV fistula to be taken only after consent from patient's physician.

10.7.2. Arm containing I.V. drip:

10.7.2.1. Prefer opposite arm

10.7.2.2. If not possible in opposite arm, I.V. drip should be shut for at least 3 minutes before a

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sample is obtained. Sample to be taken below infusion site.

10.7.2.3. Haematoma and scarring: Arm containing haematoma and scarring should be avoided.

If not possible, then select site distal to haematoma.

10.7.2.4. Mastectomy:

Single mastectomy: Arm on the side of mastectomy is avoided because surgery may have caused lymphostasis on that side.

Double mastectomy: Arm on which mastectomy has been done earlier is selected.

If both side mastectomy is done within six months, vein on the back of hand or at the ankle is selected.

11. Gloves:

The personnel responsible for collection of sample will wear gloves before venipuncture. Exposure to latex can rarely trigger a life-threatening reaction so it is vital that no latex items should be used on a latex-sensitive patient or even brought into the room. Patient should be asked about latex allergy if present latex free gloves to be worn.

12. Venipuncture:

12.1. Cleaning the venipuncture site:

12.1.1. Responsibility: Technician

12.1.2. It should be cleansed before every venipuncture.

12.1.3. Method:

12.1.3.1. The area around the intended puncture site will be cleansed with prepackaged 70 % isopropyl alcohol swab.

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12.1.3.2. Cleaning should be done in a circular motion from the site of venipuncture towards outside.

12.1.3.3. The skin should be allowed to dry. After cleaning, the skin should not be touched until venipuncture has been performed.

12.1.3.4. No alcohol or cleanser should be allowed to remain on the skin because traces may cause hemolysis and invalidate test results.

12.1.4. Touching the site after cleansing.

If the venipuncture proves difficult and the vein to be touched again to draw blood, the site should be cleansed again as explained above.

12.1.5. Procedure of venipuncture:

Appropriate needle must be selected. Most commonly used size are 19 to 22 gauge. The larger the gauge number, smaller the bore. The usual choice for an adult with normal veins is 20 gauge; if veins tend to collapse easily, a size 21 is preferred. For volumes of blood from 30 to 50mL, an 18-gauge needle may be required to ensure adequate blood flow. All needles must be sterile, sharp and without barbs. If blood is drawn for trace element measurements, the needle used are made of stainless steel and should be free from contamination.

Step 1. First, a needle or winged (butterfly) set is screwed into the collection tube holder.

Step 2. After the skin has been cleaned, the needle should be guided gently into the patient's vein.

Step 3. The vacutainer tube should be gently tapped to dislodge any additive from the stopper before the needle is inserted into a vein; this prevents aspiration of the additive into the patient's vein.

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Step 4. Once the needle is in place, the tube should be pressed forward into the holder to puncture the stopper and release the vacuum. As soon as blood begins to flow into the tube, the tourniquet should be released without moving the needle. The tube is filled until the vacuum is exhausted.

It is critically important that the evacuated tube be filled completely because many additives are provided in the tube in a ratio based on a full collection of blood; deviation or short draws can be a source of pre-analytical error because they can significantly affect test results. Once the tube is filled completely, it should be withdrawn from the holder, mixed gently by inversion eight times and replaced by another tube, if required. Avoid mixing vigorously. Other tubes should be filled using the same technique with the holder in place.

12.1.6. Venipuncture procedure using syringe:

12.1.6.1. Syringes are customarily used for patients with difficult veins. If a syringe is used, the needle is placed firmly over the nozzle of the syringe, and the cover of the needle is removed. If the syringe has an eccentric nozzle, the needle should be arranged with the nozzle downward but the bevel of the needle upward.

12.1.6.2. The syringe and the needle should be aligned with the vein to be entered and the needle pushed into the vein at an angle to the skin of approximately 15 degrees.

When the initial resistance of the vein wall is overcome as it is pierced, forward pressure on the syringe is eased, and the blood is withdrawn by gently pulling back the plunger of the syringe.

12.1.6.3. When a second syringe is necessary, a gauze pad may be placed under the hub of the needle to absorb the spill; the first syringe is then quickly disconnected, and the

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second put in place to continue the blood draw.

12.1.6.4. After removal of the needle from the syringe, drawn blood should be quickly transferred by gentle ejection into tubes prepared for its receipt or promptly analysed in the case of blood gases. The tubes should be capped and gently mixed.

12.1.6.5. Vigorous withdrawal of blood into a syringe during collection or forceful transfer from the syringe to the receiving vessel may cause hemolysis of blood sample.

12.1.6.6. Hemolysis is usually less when blood is drawn through a small-bore needle than when a larger-bore needle is used.

12.1.6.7. The needle and the holder unit is discarded into the sharps container as a unit.

12.2. When Blood collection is insufficient or no blood is withdrawn:

12.2.1. Move the syringe slightly forward (it may not be inside the lumen) or backward (it may have passed through the vein).

12.2.2. Adjust the angle of the device (the bevel should face to the upper wall of vein).

12.2.3. Loosen the tourniquet (It may be too tight and block the blood flow).

12.2.4. Try with another tube (the first one may have lost vacuum/defective).

12.2.5. Anchor the vein again (Veins sometimes move away from the tip of the syringe and the puncture site.)

12.3. Central Venous Line:

It is used primarily to administer fluids and medications, monitor blood pressure and draw blood for lab tests. Most CVADs are routinely flushed with heparin or saline to prevent clotting and a 5ml of blood must be withdrawn from the line and discarded prior to blood specimen collection. General order of sample collection is followed.

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13. Order of draw:

13.1. The recommended order of collection is as follows:

1. Blood culture bottle
2. Citrate tube (Blue cap)
3. **Plain/Gel tube (Red/yellow)**
4. Heparin tube (Green)
5. EDTA tube (Purple cap)
6. **Fluoride tube (Grey cap)**

14. Mixing of blood:

Additive tubes must be mixed immediately after collection by inverting them from three to eight times depending on the additive type.

Stopper colour	Number of inversions
Citrate tube(Blue cap)	3-4
Plain/Gel tube(Red/yellow)	5
Heparin tube(Green)	8
EDTA tube(Purple cap)	8
Fluoride tube(Grey cap)	8

If tubes are not quickly and thoroughly mixed, microclots can form in anticoagulant tubes

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and clotting may be incomplete in clot activator tubes. Gentle inversion is essential as vigorous mixing can cause hemolysis. Some tests including serum potassium, magnesium and most enzyme tests cannot be performed on hemolyzed specimens.

15. Needle stick injury:

- 15.1. Wash the wound with soap and water.
- 15.2. Do not squeeze or suck the site.
- 15.3. Inform supervisor in duty and initiate injury reporting system in the workplace.
- 15.4. Identify the source patient, who should be tested for HIV, Hepatitis B, Hepatitis C infections. The process to test the patient to be started after seeking consent.
- 15.5. Report to the SR in-charge of ER-4, NEB and then to the CMO in-charge, Room no-12 for countersigning. The first dose of prophylaxis is given by the nurses in casualty department. He is followed up in Medicine OPD.
- 15.6. Get tested immediately with confidentiality for HIV, Hepatitis B, Hepatitis C infections.
- 15.7. Get postexposure prophylactic treatment in accordance with US Centers for Disease Control and Prevention (CDC) guidelines/risk assessment when the source patient is unknown or tested positive.

16. Release the tourniquet:

As soon as the blood flow is established, tourniquet is released and the patient is asked to open the fist to allow venous flow to normalize. A tourniquet must not be left for longer

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than a minute as it affects the specimen quality.

17. Place the swab:

When the blood collection is complete and the needle is withdrawn, the patient is instructed to hold a dry clean gauze pad over the venipuncture site with the arm raised to decrease the chance of leakage of blood.

18. Remove and dispose gauze:

The gauze applied is removed and is discarded in yellow hazardous waste bag as per BMW Guidelines 2016, (Amendment rules 2018,2019).

19. Bandage the arm:

Bandaging materials are used to cover the site after collection, but should not be used on infants under two years of age due to the danger of aspiration.

19.1. Normal condition:

A new pad is subsequently held in place by a bandage, which is removed after 15 minutes after checking for any bleeding.

19.2. Continuous bleeding:

Some patients (eg. Patients on anticoagulant therapy) take longer than normal time to stop bleeding. In such condition, pressure is to be applied to the site until the bleeding stops. If the bleeding continues beyond 5 minutes, the Senior Resident or Doctor on Duty has to be notified.

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20. Label blood collection tubes and record the time of collection:

After collection of blood, the blood collection tubes are labelled with the name of the patient, age and sex of the patient, MRD no., ward/OPD name, date and time of the collection. The manufacturer's tube label can be inscribed with the patient's complete information. **The laboratory documents the time when the specimen was collected. ***

21. Transportation of blood collection tubes to lab:

21.1. From OPD Sample Collection room to Laboratory Reception.

21.1.1. The samples are collected by the trained person in Room no.19, Central Collection Lab Of OPD building marked with patient name, MRD number and unique lab number. The requisition slip for tests to be performed is also checked and collected along with the samples.

21.1.2. The Nursing Attendant from Routine Biochemistry Lab will collect the samples in **steel transport box containing ice packs*** and transport them hourly to the Lab. All the Nursing Attendants involved in sample transportation should put on disposable latex rubber gloves, and apron to protect against any type of infection.

21.2. From IPD wards / floors to Laboratory Reception.

21.2.1. The samples are collected by the Nursing Attendant of the respective wards and transported to the Routine Biochemistry Lab in **sample transport box***. All the Nursing Attendants involved in sample transportation should put on disposable latex rubber gloves, and apron to protect against any type of infection.

21.2.2. The samples are received everyday during 8.00-11.30AM after checking the requisition slip for the patient's details, tests to be performed and the Blood Collection Tubes for

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the patient's details of name, IPD number, tests requested.

21.2.3.The samples are then allotted a unique Lab number by the technician of the Lab after checking the samples.

22. Sample processing

As the samples are received in the clinical Biochemistry lab from the wards/central collections/Clinical Biochemistry collections/Super Speciality Block collections, their identity in the requisition slips and in the vacutainers are ascertained first. After that samples are segregated by the designated lab technicians/attendants on the basis of whether the requisitions are combined or individual requisitions and then forwarded to the corresponding seats for further processing.

In the corresponding seats once again sample identity is confirmed and then samples are assigned lab serial numbers. An entry is made in the reporting register and then the samples are forwarded for centrifugation. Centrifugation is done at 2200-2500 RPM for at least 5-10 minutes. Samples are then arranged according to lab serial numbers in the racks and then forwarded for analysis.

Once analysis is done print outs from the equipment are taken out and then entries are made in the corresponding reporting registers and requisition slips.

All critical values, hemolyzed samples, lipemic, QNS samples are informed telephonically to

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the corresponding wards immediately and record is maintained in the critical value register and sample rejection register respectively.

Then requisition slips are sent to the faculties/doctor on duty for signature. Reports are then sent to the corresponding wards/OPDs.

In case of urgent sample request:

In Emergency cases, the samples along with requisition slip are checked and received by the Senior Resident on duty. They are notified specially on the requisition form. Then, the samples are handed over to the concerned Senior Technician for further processing. Those samples are run in priority basis and reported immediately to the Doctor on Duty/Staff on Duty of the corresponding wards.

23. Sample storage

Samples are preserved at 2-8°C for 24 hours.

24. Sample disposal

Samples are discarded as per the Safdarjung Hospital policy and Biomedical Waste Management rules 2016 (Amendment rules 2018, 2019) after 24 hours.

25. Urine specimen collection

25.1. Collection and transport guidelines:

25.1.1. Collection of urine:

Urine specimen container: A clean, dry, leak proof, trace element, particle-free

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container with tight fitting lids preferably made of clear disposable material which is inert to urinary constituents. The specimen containers should not be re-used.

25.1.2. Container Labels:

25.1.2.1.The container is fixed with the label that will adhere during refrigeration or freezing.

The label on the smaller container must be placed on the container itself, not the lid or cap. The label contains patient identification which include patient's name, MRD number, date and time of specimen collection, and name of the preservative in container when applicable.

25.1.2.2.When a timed collection is complete, the specimen is delivered without delay to clinical laboratory where volume will be measured. This is done by using graduated cylinders. The volume is measured in milliliters.

25.1.2.3.Before specimen is transferred into small containers (which contains the same identifiers) it is thoroughly mixed to ensure homogeneity because composition of urine vary throughout the collection period.

25.1.2.4.If there is any delay (>2 hours) in processing of specimen, it will be preserved in refrigerator.

25.1.3. Types of collection:

25.1.3.1.First morning specimen:

It is collected immediately after patient wakeup in morning. It is the most concentrated urine of the day as it was retained in bladder for approximately 8 hours.

25.1.3.2.Random samples:

Random urine samples are untimed samples, collected in the sterilized containers without preservatives by the patient. The patients are asked to collect midstream sample in the sterilized containers. The actual time of collection (voiding) should be

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recorded on the specimen container.

25.1.3.3.24hr Urine samples:

24 hrs. urine samples for quantitative analysis, are collected in the sterilized containers with appropriate preservatives by the patient. Preservatives are put in the container prior to the collection. On the day of collection, 1st morning sample is discarded, then collection is done for the next 24 hours, till the 1st morning sample of the next day. During the collection, the container has to be kept in a cool, dry place to prevent possible growth of microorganisms.

25.1.4. Refrigeration:

For specimens that cannot be analyzed within two hours of collection, it is preserved by refrigeration. Refrigeration for periods longer than 24-hours may compromise the stability of some analytes. Therefore, it is best to deliver urine specimens to the testing area as soon as they are received.

25.1.5. Collection of urine from children:

25.1.5.1. To collect an untimed urine specimen from a child, the penis and scrotal or perineal area is first cleaned and dried, to remove any natural or applied skin oils.

25.1.5.2. The collection of specimens from older children is done as in adults, using assistance from a parent when it is necessary.

26. Collection of fluids

Pleural/peritoneal/ synovial/ pericardial fluids is collected in sterilized containers by the

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clinicians and sent to laboratory.

27. Criteria for Rejection of samples

1. Unlabelled or mislabelled samples- if the samples are not labelled with either patient's name, UHID no., ward or OPD location, then it must be rejected.
2. Duplicate samples- If it has been verified by the person receiving the sample, that two samples received at the same time are identical, then one of these samples may be used and if insufficient quantity in both containers, then they can be combined and processed as one.
3. Inappropriate samples- samples received in wrong vacutainer should be discarded.
4. Grossly hemolyzed
5. Highly lipaemic samples
6. QNS (Quantity not sufficient)

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