संजय गाँधी स्नातकोत्तर आयुर्विज्ञान संस्थान

Sanjay Gandhi Postgraduate Institute of Medical Sciences

# STANDARD OPERATING PROCEDURES (SOPs) FOR LABORATORIES

**Sanjay Gandhi Post Graduate Institute of Medical Sciences**
**Lucknow**

Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**SOP for Laboratory,**
**Ist Edition: February; 2023**
**Quality Assurance Cell**
**Sanjay Gandhi Post Graduate Institute of Medical Sciences**
**Lucknow, U. P. – 226014**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**DETAILS OF THE DOCUMENT**

----------**HOSPITAL**

Address: _____

| | |
|---|---|
| **Document Name :** | |
| **Document No. :** | |
| **No. of Pages :** | |
| **Date Created :** | |
| **Prepared By :** | **Designation :**<br>**Name :**<br>**Signature :** |
| **Approved By :** | **Designation :**<br>**Name :**<br>**Signature :** |
| **Responsibility of Updating :** | **Designation :**<br>**Name :**<br>**Signature :** |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# Index





Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## AMENDMENT SHEET

| S. No. | Page no. | Date of amendment | Details of the amendment | Reasons | Signature of the reviewing authority | Signature of the approval authority |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## CONTROL OF THE DOCUMENT

The holder of the copy of this manual is responsible for maintaining it in good and safe condition and in a readily identifiable and retrievable form.

The holder of the copy of this manual shall maintain it in current status by inserting latest amendments as and when the amended versions are received.

The Manual is reviewed atleast once a year (or in between SOS if so required) and is updated as relevant to the Hospital policies and procedures.

The Authority over control of this manual is as follow:

| **Prepared By** | **Approved By** | **Issued By** |
|---|---|---|
| Name:<br>Designation: HOD /Dept. In charge<br><br>Signature: | Medical Superintendent<br>Name:<br><br>Signature: | Quality – Nodal Officer<br><br>Name:<br><br>Signature: |

The Original Procedure Manual with Signatures on the Title page is considered as **"Master Copy"**, and the photocopies of the master copy for the distribution are considered as **"Controlled Copy"**.

Distribution List of the Manual

| Sr. No. | Officials | Signature of Officials receiving copy |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 1-LABORATORY COLLECTION OF BLOOD SAMPLES

**Purpose**: Collection and Handling of Blood Samples

**Scope**: To define the blood collection procedures for all sections including Haematology, Biochemistry, Microbiology and Serology

**Responsibility**: Lab In – charge/ Pathologist/ Microbiologist/ Biochemist

**Procedure**: as under

| | Activity/ Description | Responsibility | Ref. Doc. Record |
|---|---|---|---|
| | **1.4.1 Blood specimen collection and handling**<br><br>Each request for a blood specimen / any type of sample collection should be accessioned to identify all paperwork and supplies associated with each patient<br>● Accession is in the form of entry in the Out Patient Sample Collection Register by the registration assistants and affixing a number on the request form.<br>● For specimens other than blood, appropriate containers should be labeled and given to the patients with proper instructions for collection and handling of specimens.<br>● For blood collection, the patients are directed to collection counters according to the accession numbers which is displayed. | Technical Staff posted at Collection Counter | |
| | **1.4.2 Approaches and Identify the Patient**<br><br>**1. Out Patient Sample Collection**<br>The technician should identify himself or herself, establish a rapport, and gain the patient's confidence. The technician must NOT perform blood collection against the patient's or guardian's verbal consent.<br>**2. In Patient Sample Collection**<br>The doctor / paramedical staff / nurse/technicians responsible for collection of sample. He/she shall | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

identify himself or herself, establish a rapport, and gain the patient's confidence.

**3. Identify Patient**

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.

**a) 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.

**b) 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. A gauze pad should be readily available and the tourniquet is quickly released in the event the needle is violently removedor repositioned. If the needle goes accidentally muchdeeper into the arm, inform the doctor's / nurse's station.

**c) 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.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | 2. Compare the data with the information on the patient's chart or the request form.<br>3. In case of any discrepancy, report to the senior / In charge / doctor for clarification.<br><br>d) **Procedure for Identifying Unidentified Emergency Patients ***<br>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. | | |
| | 4.3 **Verify patient Diet Restrictions**<br>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.(see annexure) Biochemist to add | Technical Staff posted at Collection Counter | |
| | **Assemble Supplies**<br>The following supplies should be available at any location where Venipuncture is performed routinely:<br>• Blood Collection tubes / blood culture bottles<br>• Needle<br>• Single-use tube / needle holder<br>• Syringe<br>• A tourniquet<br>• Alcohol prep pads<br>• 1 to 10% Povidone-iodine pads, tincture iodine, or chlorhexidine compounds if blood culture is to be drawn<br>• adhesive ANTISEPTIC bandages<br>• Gloves<br>• Sharps Container/ needle destroyer as per availability in the hospital<br>• Dustbin as per colour code<br>• Blood Mixer (subject to availability) | | |
| | **1.4.5 Position Patient**<br><br>**1. Procedure for seating patient**<br>• 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.<br>• Have the patient position his / her arm rested in a comfortable position and extend the arm to form | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | a straight line from the shoulder to the wrist. Arm should not be significantly bent at the elbow.<br><br>**2. Procedure for patient lying supine**<br>• Ask the patient to lie down on his / her back in a comfortable position.<br>• If additional support is needed, place a pillow under the arm from which the specimen is being drawn.<br>• Have the patient position his / her arm extends to form a straight line from the shoulder to the wrist. | | |
| | **1.4.6 Apply Tourniquet**<br>A tourniquet is used to increase venous filling. This makes the vein more prominent and easier to enter.<br>**1. Precautions while using tourniquet**<br>Tourniquet application should not exceed one minute as localized stasis with haemoconcentration 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.<br>**2. Tourniquet location**<br>Wrap the tourniquet around the arm 3 to 4 inches above the Venipuncture site.<br>**3. Blood Pressure cuff**<br>If a blood pressure cuff is used as a tourniquet, inflate it to 40mm Hg.<br>**4. Ensure patient's hand is closed**<br>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.<br>**5. Select Vein**<br>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.<br>**Factors to avoid in site selection**<br>**a. Extensive scarring**<br><br>**b. Mastectomy**<br>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. | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **c. Hematoma**<br>Phlebotomy should not be performed on any size hematoma. If another vein site is not available, the specimen is collected distal to the hematoma.<br><br>**d. Intravenous therapy**<br>Preferably, specimens should not be collected from an arm with an intravenous site.<br><br>**e. Cannula, Fistula, Vascular Graft**<br>A cannulated arm is used only after consulting attending physician.<br><br>**7. Procedure for Vein selection**<br>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. | | |
| | **1.4.7 Put on Gloves**<br>The phlebotomist must wear gloves (sterile/unsterile as per the availability) before the Venipuncture is performed. | Technical Staff posted at Collection Counter | |
| | **1.4.8 Cleanse Venipuncture Site**<br>The puncture site must be cleansed<br><br>**1. Cleansing Method for Venipuncture**<br>• Use a gauze pad/swab with 70% isopropyl alcohol solution.<br>• Cleanse the site with a circular motion from the centre to the periphery.<br>• 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.<br><br>**2. For Blood Culture Collection**<br>• For blood cultures, it is necessary to carefully | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | disinfect the Venipuncture site.<br>• Chlorhexidine Gluconate is recommended for infants two months and older and patients with iodine sensitivity.<br>• Cleanse the site with 70% alcohol, then swab concentrically, starting at the middle of thesite with a 1 to 10% povidone-iodine solution (0.1 to 1% available iodine) / or chlorhexidine gluconate.<br>• Allow the site to air dry and then remove the iodine or chlorhexidine from the skin with alcohol.<br>• When specimens are obtained for blood cultures, disinfect the culture bottle stopper according to the manufacturer's instructions.<br><br>**3. Touching the Site after Cleansing**<br>If the Venipuncture proves difficult and the vein must be touched again to draw blood, the site should be cleansed again. | | |
| | 1.4.8.1 **Perform Venipuncture**<br><br>**1. Venipuncture Procedure When Venous Blood Collection Tubes Are Used**<br>There are several different blood collections systems available that collect blood samples using different principles.<br>• Thread the appropriate needle into the holder until it is secure.<br>• When drawing blood for cultures, wipe the stopper with a suitable antiseptic solution. Make certain the stopper is dry before performing the Venipuncture.<br>• Make sure the patient's arm or other Venipuncture site is in a downward position to prevent reflux of "backflow".<br>• 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.<br>• To prepare the patient, inform him or her that the Venipuncture is about to occur.<br>• With the bevel up, puncture the vein with the needle at an angle of insertion of 30 degrees of less. Keeping the needle as stable as possible in the vein, pushy/connect the first tube onto the | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

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. During the collection, do not allow the contents of the tube to contact the closure. Movement of the blood back and forth can cause reflux into the venous system and possible adverse patient reaction.
- 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.
- 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 isinserted / connected to the needle/holder. To obtain additional specimens, insert/connect the next tube to the needle/holder prior towithdrawing the needle from the vein. If onlyone 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 five to ten times. To avoid hemolysis, do not mix vigorously.

**2. Venipuncture Procedure Using Needle and Syringe**

In general, Venipuncture using a needle and 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.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- 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.

**3. Fill the Tubes If Syringe and Needle are Used**

Syringe method of drawing venous blood is no 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.
- To transfer the blood from the syringe to a venous blood collection tube by removing the stopper.
- Mix additive tubes by inversion.

**4. Blood Specimen That Cannot Be Obtained**

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 basilicvein, since nerves and the brachial artery are in close proximity.
- Try another tube to ensure the tube selected is not defective.
- 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.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **5. Ensure Patient's Hand Is Open**<br>Opening the patient's hand reduces the amount of venous pressure as muscles relax. The patient must not allowed to pump the hand. | | **Finger prick/Heel Prick Procedure- See Annexure 1** |
| | **1.4.9 Order of Draw**<br><br>**1. Plastic Venous Blood Collection Tubes**<br>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. These procedures should be followed for venous blood collection tubes. Thetubes should be mixed by inversion gently.<br>• Blood culture tube (8 to 10 times)<br>• Coagulation tube (blue top) (3-4 Times)<br>• Serum tube with or without clot activator, with or without gel (red top/yellow top) (5 times)<br>• Heparin tube (green top) (8-10 times)<br>• EDTA (lavender top) (8-10 times)<br>• Glycolytic inhibitor (gray top) (8-10 times)<br><br>**2. Coagulation Testing**<br>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 istaken, 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. | | |
| | **1.4.10 Releases the Tourniquet**<br>Release the tourniquet as soon as possible after the blood begins to flow. | | |
| | **1.4.11 Place the Gauze Pad/Swab**<br>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. | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **1.4.12 Remove and Dispose of the Needle**<br>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. | Technical Staff posted at Collection Counter | |
| | **1.4.13 Bandages the Arm**<br>**Normal Conditions**<br>Under normal conditions:<br>1. Place the gauze pad / cotton ball over the site, continuing mild pressure.<br>2. Check that bleeding has ceased, observe for hematoma.<br>3. Tell patient to leave the pad / cotton ball on by applying mild pressure for at least 15 minutes.<br><br>**Continued Bleeding**<br>1. Watch for excessive bleeding.<br>2. If a hematoma develops of bleeding persists longer than five minutes, a nurse 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.<br>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. | Technical Staff posted at Collection Counter | |
| | **1.4.14 Label Blood Collection Tubes and Record Time of Collection**<br>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.<br>• The patient's first and last name<br>• Age/sex<br>• An identification number<br>• The date<br>• The time (as required e.g. therapeutic monitoring)<br>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. | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | 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. | | |
| | **1.4.15 Specimens that need transport at cool temperature**<br>Certain tests require that blood specimens be cooled immediately following the Venipuncture are and transport with ice packs:<br>• Ammonia<br>• Lactic acid<br>• Blood gas analysis<br>• Parathyroid (PTH) hormone | Technical Staff posted at Collection Counter | |
| | **1.4.16 Transportation of primary sample with specification about time frame, temperature and carrier** | | |
| | **From IPD wards / floors to LaboratoryReception**<br>• The samples are collected by the TRAINED PERSONNEL<br>• The specimens are checked that the proper identification of the patient (name, IPD no.) and the tests to be performed are marked.<br>• It is verified that the specimen are OK for testing as per SOP Rejection of primary sample<br>• Once the specimen is OK it is allotted a unique identification lab number and the steps as per Above are followed. | Nursing Sister Incharge | |
| | 1.4.16.2 **From OPD Sample Collection room to Laboratory Reception**<br>• The samples are collected and properly marked with the patient name and its unique identification lab number.<br>• To check the slip is attached for the tests to be performed on each specimen with the time of draw.<br>• All specimens are kept in the box / tray and transported to lab within 30 minutes.<br>• The specimen that needs to be processed | Technical Staff posted in Collection area | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | immediately and for urgent report, they are sent to lab as soon as they are collected. | | |
| | 1.4.16.3 **Specimens that need transport at cool temperature**<br>Certain tests require that blood specimens be cooled immediately following the Venipuncture are and transport with ice packs:<br>• Ammonia<br>• Lactic acid<br>• Blood gas analysis<br>• Parathyroid (PTH)hormone | Nursing Sister Incharge in consultation with the respective Labs | |
| | **1.4.17 Rejection of primary samples**<br>• Mislabelled/Unlabelled specimens<br>• Improper container<br>• Quantity not sufficient for testing<br>• Without test request<br>• Haemolysed<br>• Clotted (Where not indicated)<br>Samples not adhering to the Vaccum blood collection tubes specifications | Technical Staff Counter checked by Lab Incharge | |
| | **1.4.18 Receipt, Labeling, Processing and Reporting of primary sample** | Laboratory Technical Staff | |
| | To receive a complete requisition form W.R.T<br>a) Name of the physician or other person legally authorized to make request for examination<br>b) Type of primary sample and the anatomic site of origin.<br>c) Examination requested.<br>d) Clinical information of the patient for interpretation purpose<br>e) Date and time of receipt of sample in the laboratory. | | |
| | **1.4.19 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) | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **1.4.20 Receipt, Labelling, Processing and Reporting of primary sample in case of urgent sample request**<br>CRITERIA FOR URGENCY/EMERGENCY MUST BE DEFINED AT INDIVIDUAL HOSPITAL LEVEL | Technical Staff<br>Counterchecked by Lab Incharge | |
| | 1.4.20.1 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. | | |
| | 1.4.20.2 This information for urgent samples may also be communicated telephonically to the reception and respective sections of lab. | | |
| | 1.4.20.3 The specimens are checked for the proper identification as per SOP Rejection of primary samples | | |
| | 1.4.20.4 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 available ward boys or reception staff | | |
| | **1.4.21 Instructions for primary sample storage** | Technical Staff | |
| | 1.4.21.1 The examined primary samples are stored for re-examination and/or additional tests for a minimum period as specified below:<br><br>**Clinical Biochemistry:** 48 hours at 2-8$^0$C<br>**Haematology 24 hours**<br>**Serology/ Microbiology: refer to the specific SOP** | | |
| | **1.4.22 instructions for repeat testing** | Technical Staff<br>Counterchecked by Lab Incharge | |
| | 1.4.22.1 Under following conditions repeat tests are performed:<br>**BIOCHEMISTRY:**<br>1. When the results are beyond the linearity limit, sample is tested in appropriate dilution / concentration.<br>2. Highly abnormal results are retested for confirmation.<br>3. When the results do not correlate with other results. | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | 4. On request of clinicians / patient.<br>• In case of 1st, 2nd& 3rd point mentioned above, even if the result is within the close tolerance than the second reading is given in the report and both reading are mentioned in the internal work book. However, if the variation is significant the second reading is taken as confirmative and this result is mentioned in the report.<br>• In 4th case the result is compared with the original report and the fresh report is given with the current reading.<br>5. HAEMATOLOGY (Disparity between cell counter and microscopic findings)<br>CLINICAL PATHOLOGY/ FLUIDS – for confirmation of malignancy and any atypical finding | | |
| | **1.4.23 Processs Efficiency Criteria OPD**<br>• Less than 1% repeat sampling*<br>• Less than 1% haemolysed samples*<br>• Haematoma<br>• Blood Spills<br>• Needle Stick Injury<br>• Patient Complaints<br>• Wrong Labelling<br>*Each hospital may define their own criteria. | Senior Most Technical Staff<br>Monitored by the Lab Incharge | Should be Documented in the incident register/Complaint /Grievance Register |
| | **1.4.24 Processs Efficiency Criteria IPD**<br>1. Wrong Labelling<br>2. Less than 1% repeat sampling*<br>3. Less than 1% haemolysed samples*<br>4. Haematoma<br>5. Blood Spills<br>6. Needle Stick Injury<br>7. Patient Complaints<br>*Each hospital may define their own criteria. | Sister Incharge<br>Doctor On Duty<br>Resident doctor incharge<br>Senior Most Technical Staff (1-3) will communicate to the sister in charge Ward/IPD<br>Monitored by the Lab Incharge | Should be Documented in the incident register/Complaint /Grievance Register |

**Note: Preferably HIMS system should be in place. These SOP are meant for the centers where the bar coding facility is not available.**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## Annexure

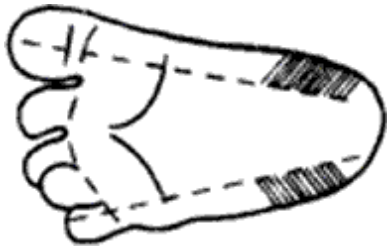
## 1 Sample collection (blood)

### 1. Finger stick 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.
- Massage the finger toward the selected site prior to the puncture.
- Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. 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.
- Cap, rotate 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.

### 2. Heel stick 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.





Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
- 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.

## 3. 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.
- 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.

## 4. Blood Sample Handling and Processing:

Pre-centrifugation Handling –

- The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:
  - Fill 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.
  - Blood vacuum tubes should be stored at 4-25°C (39-77°F).

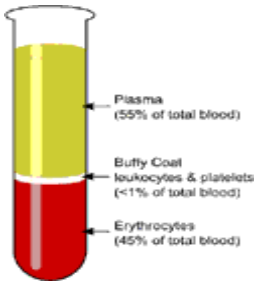


Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Tubes should not be used beyond the designated expiration date.
- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

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.




- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a “balanced load” noting the following:
- Opposing tube holders must be identical and contain the same cushion or none at all.
- Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
- If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Safety-

- Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.
- Centrifuges must never be operated without a cover in place.
- Uncovered specimen tubes must not be centrifuged.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
- Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.
    - Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 2. CLINICAL PATHOLOGY

**Purpose**: Collection and Handling of Urine Samples, Stool samples or Semen Samples

**Scope**: to define the urine/Stool/Semen collection procedures for all sections including Biochemistry, Microbiology and Clinical Pathology

**Responsibility**: Lab Incharge/ Pathologist/Microbiologist/Biochemist

**Procedure**: as under

| | **Activity/ Description** | **Responsibility** | **Ref. Doc. / Record** |
|---|---|---|---|
| | **2.4.1 Specimen collection and handling**<br><br>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<br><br>● Accession is in the form of entry in the Out Patient Sample Collection Register by the registration assistants and affixing a number on the request form.<br>● Appropriate containers should be labeled and given to the patients with proper instructions for collection and handling of specimens. | Technical Staff posted at Collection Counter | |
| | 2.4.1.1 **Approaches and Identify the Patient**<br><br>**1. Out Patient Sample Collection**<br>The technician should identify himself or herself, establish a rapport, and gain the patient's confidence.<br><br>**2. In Patient Sample Collection**<br>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. | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**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.

**e) Patient who is Conscious**

The steps are as follows:

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

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

The steps are as follows:

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

**4 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:

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

5 **Procedure for Identifying Unidentified**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **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. | | |
| | 2.4.1.2 **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) | Technical Staff posted at Collection Counter | |
| | **Assemble Supplies** The following supplies should be available at any location where urine/stool/semen examination is performed routinely: • Urine culture bottles • Urine collection bottles • Appropriate container for stool and semen collection as per the annexures • Gloves • Dustbin as per colour code • Test tubes • Pasteur Pipettes • Centrifuge • Microscope • Glass slides • Cover slip | | |
| | **2.**4.1.4 **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 tubes identified with a label bearing at least the following. • The patient's first and last name • Age/sex • An identification number • The date • The time The completed label must be attached to the 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 | Technical Staff posted at Collection Counter | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | responsible for collecting the specimen shall be recorded. | | |
| | 2.4.1.5 **Send Sample Containers to Laboratory**<br>Appropriately labeled urine/stool/semen containers should be sent to laboratory designated to perform the required testing procedures. | Technical Staff posted at Collection Counter | |
| | **2.4.2 Transportation of primary sample with specification about time frame, temperature and carrier** | | |
| | **From IPD wards / floors to Laboratory Reception**<br>• The samples are collected by the TRAINED PERSONNEL<br>• The specimens are checked that the proper identification of the patient (name, IPD no.) and the tests to be performed are marked.<br>• It is verified that the specimen are OK for testing as per SOP Rejection of primary sample<br>• Once the specimen is OK it is allotted a unique identification lab number and the steps as per A above are followed. | Nursing Sister Incharge | |
| | 2.4.2.2 **From OPD Sample Collection room to Laboratory Reception**<br>• The samples are collected and properly marked with the patient name and its unique identification lab number.<br>• TO CHECK THE slip is attached for the tests to be performed on each specimen with the time of draw.<br>• All specimens are kept in the box / tray and transported to lab within 30 minutes.<br>• The specimen that needs to be processed immediately and for urgent report, they are sent to lab as soon as they are collected. | Technical Staff posted in Collection area | |
| | | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **2.4.3 Rejection of primary samples**<br><br>As per the respective annexure | Technical Staff<br>Counter checked by Lab Incharge | |
| | **2.4.4 Receipt, labeling, processing and reporting of primary sample** | Laboratory Technical Staff | |
| | To receive a complete requisition form W.R.T<br>f) Name of the physician or other person legally authorized to make request for examination<br>g) Type of primary sample and the anatomic site of origin.<br>h) Examination requested.<br>i) Clinical information of the patient for interpretation purpose<br>j) Date and time of receipt of sample in the laboratory. | | |
| | 2.4.4.2 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) | | |
| | **2.4.5 Receipt, labeling, processing and reporting of primary sample in case of urgent sample request**<br>CRITERIA FOR URGENCY/EMERGENCY MUST BE DEFINED AT INDIVIDUAL HOSPITAL LEVEL | Technical Staff<br>Counter checked by Lab Incharge | |
| | 2.4.5.1 **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 boy**s. | | |
| | 2.4.5.2 This information for urgent samples may also be communicated telephonically to the reception and respective sections of lab. | | |
| | 2.4.5.3 The specimens are checked for the proper identification as per SOP Rejection of primary samples | | |
| | 2.4.5.4 Once the specimen is found OK it is allotted a unique identification lab number | | |
| | 2.4.5.5 Then the samples are specially transported to the respective sections of the lab on priority basis by available ward boys or reception staff | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **2.4.6 Instructions for primary sample storage** | Technical Staff | |
| | 2.4.6.1 The examined primary samples are stored for re-examination and/or additional tests for a minimumperiod as specified below:<br><br>**Clinical pathology/fluids 24 hours** | | |
| | **Instructions for repeat testing**<br>6. Highly abnormal results are retested for confirmation.<br>7. When the results do not correlate with other results.<br>8. On request of clinicians / patient.<br><br>Also See respective annexure | Technical Staff<br>Counter checked by Lab Incharge | |
| | **Process Efficiency Criteria OPD**<br>• Patient Complaints<br>• Wrong Labelling<br>• Compliance with the Turn Around Time set (as under)<br>• Timely reporting and documentation of Critical Reports as per the criteria | Senior Most Technical Staff<br>Monitored by the Lab Incharge | Should be documented in the incident register/Complaint /Grievance Register |
| | **2.4.9 Process Efficiency Criteria IPD**<br>8. Patient Complaints<br>9. Compliance with the Turn Around Time set (as under)<br>10. Timely reporting and documentation of Critical Reports as per the criteria | Sister Incharge<br><br>Doctor On Duty<br><br>Resident doctor incharge<br><br>Senior MostTechnical Staff (1-3) will communicate to the sister in charge Ward/IPD<br><br>Monitored by theLab Incharge | Should be documented in the incident register/Complaint /Grievance Register |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **2.4.10 Critical reference value and procedure:**<br>**Refer to Annexure**<br>Critical values are made as per clinical critical levels of different sections of the lab. All the critical values are informed both telephonically to the concerned doctor and by critical alert movement register maintained by lab. | Technician on duty and Doctors on duty | A Critical Value movement register* is maintained by the lab to the concerned departments.<br>(*as per the individual Hospital defined criteria) |
| | **2.4.11 Release of report:**<br>All the lab reports are dispatched after final reporting by the lab doctor. The turn around time of the lab reports are<br>• Urine/stool/Semen on same day for IPD and next day for OPD<br>• Emergency reports released on same daywithin 2 hours. | Technical Staff on duty | Dispatch registeris maintained.<br>Emergency sample register is maintained. |
| | **2.4.12 Internal Quality control system**<br>It is a regular process carried out in the lab to standardise the lab system of all sections by-<br>1. By running internal quality control (commercially available) routinely, at the beginning of processing of all the routine samples in case of automated urine analysers<br>2. Repeat runs on the random samples<br>3. Matching the machine report with microscopic report.<br>4. split sampling | Senior Technician supervised by Department Incharge | Internal quality control registers are maintained. |
| | **2.4.13 External quality control system**<br>1. Split Sampling<br>2. Inter Laboratory Comparison | Senior Technician supervised by Department Incharge | External quality control register and file is maintained. |
| | **2.4.14 Calibrations of equipment**<br>• All the lab equipments are to be calibrated periodically by the company engineer.(six monthly) | Senior Technician supervised by Department Incharge | A log book and files are to be maintained. |
| | **2.4.15 Validation of reagents, stains, kits etc:**<br>• Random re -sampling<br>• By checking the Lot ,batch no ,and expiry date .<br>• Cross checking of the slides for microscopic findings.<br>• For validation of stains, control slides are checked. | Senior Technician supervised by Department Incharge | Quality Control Register to be maintained. |
| | **2.4.16 System of resolution of complaints and feedbacks from stakeholder.**<br>Any lab related complaint and feedbacks are to be resolved by the department incharge and patient | Senior Technician supervised by Department Incharge | Complaints and feedback register is maintained |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | satisfaction should be the priority. | | |
| | **2.4.17 Referral laboratories (as per the State Government Policy)** | | |
| | **2.4.18 Storage, retaining and retrieval**<br>Priority should be given to timely dispatch of the reports as per the Turn Around Time.<br>All uncollected/unclaimed Lab reports are to be stored for 1 week only<br><br>**Preferably HIMS system should be in place**<br><br>*Storage of samples*<br>Primary and examination samples<br>• Clinical Pathology samples 24 hours. | Senior Technician supervised by Department Incharge<br>To be Communicated to the concerned OPD/IPD incharge | Dispatch registers to be maintained Each hospital to set their own dispatch procedure |
| | **2.4.19 Control of documents**<br>• Random checking of the documents<br>• Checking the page numbering<br>• Signing of the documents<br>• Removal of obsolete documents and<br>• Any modification to be done | Senior Technician supervised by Department Incharge | Register maintained. |
| | **2.4.20 Procedure for preventive and break down maintenance**<br>• Separate system for collection and handling of sample.<br>• Urgent / instant sampling preferred.<br>• Urgent reporting and dispatching .<br>• Separate register is maintained .<br>• Consultation with treating doctor if required.<br>• Regular information to the Nodal officer dealing epidemic breakdown, through documentation. | Senior Technician supervised by Department Incharge | Separate register for epidemic or breakdown is to be maintained. |
| | **2.4.21 Procedure for internal audit**<br>• All lab stock registers with page numbering are checked time to time.<br>• Lots, batch no of the kits, Date of Expiry and reagents are checked time to time.<br>• FIFO for reagents (first in first out) system is followed. *as per the available stock<br>• All the existing equipments/ machine/ non consumables are checked time to time and tally with the register. | Lab store incharge and Department incharge. | Stock register is maintained. |
| | **2.4.22 Procedures for purchase of external services and supplies**<br><br>As per the hospital procedure and policy. | Purchase Section | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# Annexure

**1. Sample collection for lipid profile**

- The test (CHOLESTEROL) may be measured any time of the day without fasting.
- However, if the test is drawn as part of a total lipid profile, it requires a 12-hour fast (no food or drink, except water).
- For the most accurate results, wait at least two months after a heart attack, surgery, infection, injury or pregnancy to check levels.
- Avoid coffee at least one day before specimen collection.

**Glucose Tolerance Test (GTT) or The three hour glucose tolerance test**

- Patient should be instructed to maintain a high carbohydrate diet for three days prior to testing.

**Fasting is necessary before these tests:**

1. Fasting Plasma Sugar estimation – overnight fasting minimum 6 to 8 hrs.
2. Lipid Profile/ Cholesterol/ Triglycerides/ HDLC/ LDLC estimation.
3. Serum Iron Studies Iron/TIBC – overnight fasting 6 to 8 hrs.
4. All samples for hormone assays should preferably be collected under fasting conditions

Fasting implies NO tea, coffee, milk, breakfast, juices, fruits, medications till the blood sample is collected. WATER consumption is ALLOWED at any time before the sample collection, preferably in regular sips.

**Annexure for clinical pathology**

**Stool sample collection for occult blood**

Patients should be instructed to eat a well balanced diet including fiber such as bran cereals, fruits and vegetables. Avoid the following for three days before and during the stool collection period:

- Red meats (beef, lamb, and liver)
- Vitamin C in excess of 250 mg a day from supplements, citrus fruits juices
- High peroxidase containing fruits and vegetables including turnips, horseradishes, radishes, broccoli, cauliflower and cantaloupes

## 1. Semen analysis

### Indications of seminal fluid examination

1. useful in detecting male infertility
2. useful for ascertaining the effectiveness of vasectomy
3. for support or denial of paternity on grounds of sterility
4. in forensic studies



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

5. To detect any marked degree of abnormal forms and for a genetically defective embryo, in cases of habitual abortion.

## Collection of specimen

- It is usually recommended that the semen sample be collected following a three-day period of abstinence. (a minimum of 2 days and a maximum of 7 days of sexual abstinence)
- If additional samples are required, the number of days of sexual abstinence should be as constant as possible at each visit.
- The person should be given clear written and spoken instructions concerning the collection of the semen sample.
- These should emphasize that the semen sample needs to be complete and that the person should report any loss of any fraction of the sample.
- The most satisfactory specimen is that which is collected in the clinical pathology laboratory by masturbation.
- Acceptable, but somewhat less satisfactory, are the specimens obtained in the patient's home by coitus interruptus or masturbation and delivered soon thereafter to the laboratory.
- The specimen may be collected in clean and dry wide mouthed, glass, polythene or plastic containers. The containers must be free from detergents or harmful contaminants
- Condom collection is not considered ideal because the powder or lubricant applied to the condoms may be actively spermicidal.
- The specimen container should be kept at ambient temperature, between 20 °C and 37 °C, to avoid large changes in temperature that may affect the spermatozoa after they are ejaculated into it.
- Sterile collection of semen for microbiological analysis- In this situation, microbiological contamination from non-semen sources (e.g. commensal organisms from the skin) must be avoided. The specimen containers, pipette tips and pipettes for mixing must be sterile. The person should: Pass urine. Wash hands and penis with soap, to reduce the risk of contamination of the specimen with commensal organisms from the skin. Rinse away thesoap. Dry hands and penis with a fresh disposable towel. Ejaculate into a sterile container.
- Time and date of collection is noted on the container along with Name and specific lab no and code no. of the hospital.

**Semen samples may contain dangerous infectious agents (e.g. human immunodeficiency virus (HIV), hepatitis viruses or herpes simplex virus) and should therefore be handled as a biohazard.**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

The specimen is to be kept in incubator until the analysis is finished. Some use water bath or dry baths for this.

**Steps to be taken at the end of one hour:**

- Liquefaction time
- Volume
- Viscosity
- Ph
- Concentration (count)
- Motility
- Viability

**Items required for semen analysis:**

- Syringes with 21g needles
- Measuring cylinder

Graduated conical tubes

- Ph paper range 6.4-8
- Glass slides
- Neubar counting chamber
- Semen diluting fluid
- 10-50 mcl micropipette / absoluter.
- Glass tubes
- Eosin stain
- Saline for washing
- Microscope

## *Physical Examination*

*Coagulation and liquefaction*

Liquefication time- after 30minutes we look for any chunks of acellular gelatinous material. If it is present then it's not completely liquified. We report it as at the end of 30minutes. Once liquefied, the sample becomes more fluid and clear. Transfer the sample to a properly labeled measuring cylinder. Normal semen sample should liquefy within one hour. In case liquefaction has not occurred, this should be noted as abnormal.

*Gross appearance*

The sample should then be examined for gross appearances such as color etc. Freshly ejaculated semen is highly viscid, opaque white or gray-white. It has a distinct musty or acrid odour. After 10-20 minutes, the coagulum spontaneously liquefies to form a translucent, turbid, viscous fluid, mildly alkaline with a pH of about 7-7.7.

*Volume*

Volume must be measured by a disposable syringe. The normal semen volume averages 3.5 ml with a usual range of 1.5-5.0 ml. Any volume less than 1 ml should be reflexly tested for fructose if possible.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

*We usually add a note to do urine examination for possible retrograde ejaculation if the semen volume is less than 1ml provided all the ejaculate is collected in the container.*

*Semen viscosity measurement:*

Take a small quantity of semen in a syringe with 21g needle and push the sample gently. Semen samples with normal viscosity should fall drop by drop. In case viscosity is increased, the sample will drop leaving a trail behind. Before viscosity is tested, ensure that the sample has liquefied.

Viscosity - checked by dropping from a pipette to see length of thread formed. We assess the viscosity by dropping using a pasteur pipette and observe for threading. If the thread is more than 2cms we consider it abnormal. We grade it [1-4].

Highly viscous semen-is rare. If it's really like gel [grade 4 viscosity] then we don't do analysis. In lesser grade of viscosity we pass the specimen many times in the pasteur pipette. If that fails to make it fluidy, then we mix equal quantities with 10% Na bicarbonate solution. But some labs employ chymotrypsin treatment.

Chemical parameters:

1. *Semen pH measurement:*

Transfer a drop of liquefied semen onto a pH paper range 6.4-8 and leave for 30 secs before reading. Normal semen should have a pH between 7.2 and 7.8. If pH is greater than 7.8, one should suspect infection and look for the presence of pus cells. If pH is less than 7 in a patient with azoospermia,
one should suspect dysgenesis of vas or seminal vesicles.

2. *Semen Fructose (Qualitative):*
   Fructose is the major sugar in seminal fluid. It is produced by the seminal vesicles. Glucose forms a very negligible component of semen. Absence of fructose in semen indicates non-development of the seminal vesicles. In our institute, we qualitatively estimate the presence or absence of fructose in the semen. Testing may be done using resorcinol, but in our lab, Benedict's Qualitative reagent is used.

   Principle: When measured quantity of semen is added to Benedict's Qualitative reagent, and the mixture heated, Fructose like glucose reduces cupric ions to cuprous ions resulting in change of color of the Benedict's reagent to shades of green to red.
   Procedure:
   1) Take 5 ml of Benedict's Qualitative reagent in a clean test tube
   2) Add 8 drops (about 0.5 ml) of semen sample using a Pasteur pipette and mix gently.
   3) Heat carefully so that the mixture boils without spillage.

   Change in color of the mixture from blue to shades of green – yellow –
   orange to red indicated presence of Fructose in the sample of semen.

## Semen – Microscopy:

**Determination of semen concentration (counts):**

Prepare 1:20 dilution of semen in a tube (50 mcl semen in 950 mcl diluting fluid 0.5% aqueous solution of sodium bicarbonate to which 1ml/100ml of neutral formaldehyde is added), and load the eubauershemocytometer. Following the liquefaction of the semen, the sperms may be counted in the haemocytometer improved Neubauer's chamber following initial dilution in a WBC pipette. Count as for WBCs in blood. After charging the chamber, two minutes are allowed for the sperms to settle. The spermatozoa in the four large squares used for WBC counting are counted. The total no. of sperms in the four squares, multiplied by 50,000 is the count of the no. of spermatozoa per ml. of seminal fluid.

If concentration is <20x106 dilution of 1:10 may be used (100mcl semen in 900 mcl diluting fluid). If concentration is greater than 100 x106 / ml, a dilution of 1:50 should be used (20mcl semen in 980 mcl diluting fluid). A rough estimate of count can be made on cover-slip preparation by taking the average count in one HPF (40x), and multiplying it by 106. This should however be used only to cross-check the counts. Counts are expressed in millions per ml. If we find no sperms on wet mount we should examine the sediment (1000xg for 10minutes) for sperms. Though ideal for counts and assessment of motility, because of high cost (~35,000 rupees) we don't use Mekler. Thenormal sperm count is 60 to 150 millions/ml.

## *Pus cells*

A simultaneous count of pus cells may be made ina wet mount using 1% Methylene blue. I have seen many labs reporting pus cells [around 10-15] in every specimen. Most of the times they are mistaking the epithelial cells as pus cells.

*Determination of semen motility:*

Dispense 10-15 mcl semen which is mixed well, onto a clean glass slide and cover slip it using a 22x22 cm cover slip. Leave the sample to settle for 1 min. Scan about 10 to 15 fields under 40x. Note the percentage of sperms having different grades of motility (a: rapid and linear progressive motility- excellent or good motility; b: slow and sluggish linear or non-linear movement - weak or moderate; c: non-progressive motility; d: immotile

Motility is classified as quick or sluggish. The degree of activity is important, since actively motile sperms lose their motility faster. Watson and Robertson have categorized motility as progressive motility, non-progressive motility, and non-motility.

In addition to motility, the presence of red cells, leucocytes, epithelial cells and autoagglutination should be observed. Autoagglutination may be seen in which the spermatozoa are attached to each other, head to head or tail-to-tail. It occurs due to autoantibody. Autoagglutination diminishes the motility of spermatozoa.

*Revitalization test*

If sperms are immobile and appear to be dead, 1 drop of glucose Ringer solution is allowed to flow under the overslip, to observe whether some of the sperms recover and become motile again.

*Sperm viability test:*

When motility of sperms is less than 40%, test for viability by the following method. Take one drop of semen (10-5mcl) on a slide and add one drop of eosin (0.5% aqueous soln). Apply 22x22 cm coverslip. Allow to stand for 2 minutes and then screen under 40x objective. Count 100 sperms and note the number of viable (unstained) and dead(stained) sperms. Lack of motility is not synonymous with mortality. Live sperms remain unstained by Eosin, while Eosin stains dead sperms.

Viability testing is done to differentiate living but immobile sperms from dead sperms. The final percentage of dead

sperms should not exceed the number of non-motile sperms counted in the motility test.

*Assessment of morphology:*

Take the air-dried smear which was prepared at the start, and stain as for blood smear by Leishman stain. Normal semen has fewer than 30% abnormal forms. A normal smear of semen contains mature sperms, sertoli cells, macrophages containing sperm heads in cytoplasm, epithelial cells, and occasionally white and red cells, and spermine hydrochloride crystals.

Count 100 sperms and note the morphologic variants of head ( normal oval, large oval, small oval, tapering head, double head, amorphous, round, pin-head) ; mid-piece (normal, cytoplasmic droplets) ; and tail piece (normal, abnormal). Normal sperms have oval head measuring 5x3 microns, with acrosomal cap covering more than 1/3 of head surface, the mid-piece is slender and less than 1/3 the width of head and measures about 7 microns in length. The tail is slender, uncoiled and measures about 45 microns in length. In normal semen sample, at least 50% of sperms should have normal morphology.

The percentage of acrosome to total head volume, middle piece and tail should be examined. Abnormal tail=abnormal sperm. Abnormal middle piece=abnormal sperm. Acrosome, <40% and >70% is abnormal. Coiled tails, angulated tails and attachment to one side are abnormal.

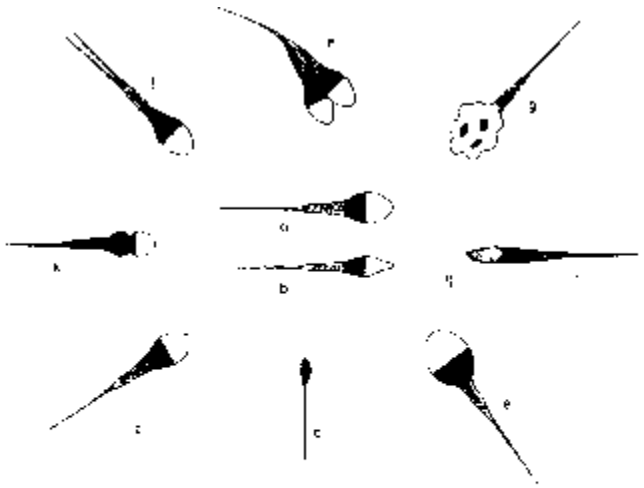


**Fig. 1** Schematic diagram of normal (a, b, c) and abnormal (d, e, f, g, h, i, j) spermatozoa: (a) Normal face view (b) Normal lateral view (c) Immature spermatozoan (spermatid) (d) Pin head (e) Giant head (f) Acute tapering form (g) Amorphous form (h) Double head (i) Double tail (j) Constricted head



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

For the purpose of morphologic description, different portions of sperm are anatomically described as head, neck and tail. Normal sperms show some variation in the shape and size of the head, e.g. small, round, pointed or enlarged. They may also show variation in staining. Variations in the morphology of the head are important. Sperm abnormalities include juvenile forms with cytoplasmic appendages, senile forms with diffuse or absent staining of the head, and pathological variations in the configuration and number of heads.

| Volume | Values |
|---|---|
| pH | 2 ml or more |
| Concentration | 7.2 to 7.8 |
| Motility | Concentration > 20 million/ml |
| Morphology | >50% with normal morphology |
| Viability | >50% live forms |
| Pus Cells | Pus cells <1mill / ml (<1x 106 /ml) |

Semen analysis – normal values, interpretation, miscellaneous

| II. Nomenclature: | |
|---|---|
| Normozoospermia | Normal ejaculate |
| Oligozoospermia | Sperm concentration < 20 million/ml |
| Asthenozoospermia | <50% with grade a & b or < 25% with grade a motility. |
| Teratozoospermia | < 50% with normal morphology |
| Oligoasthenozoospermia | Count and motility below normal |
| Oligoasthenoteratozoospermia | Count, motility and morphology below normal |
| Azoospermia | No spermatozoa in ejaculate(should be confirmed after centrifugation of the semen specimen) |
| Aspermia | No ejaculate. |
| Necrozoospermia | presence of immobile spermatozoa |

| Quantity | Ml |
|---|---|
| Turbidity | Turbid/hazy/watery |
| Viscocity | Fresh (should be coagulated) after 30 min (should be liquefied) |
| Mtility | Active % (number of actively motile sperms)<br>Sluggish % (number NOT USEFUL) |
| Viability | % (Percentage of actively motile sperms after 1 hr after collection) |
| Total Sperm Count | mill / ml |
| Morphology | Normal forms % |
| Eosin Nigresin test | Alive sperms: %<br>Dead sperms: % |
| Pus cells | / hpf |
| Epithelial cells | / hpf |
| Miscellaneous tests (Like fructose test, etc) | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

REPORT ON EXAMINATION OF SEMINAL FLUID

Specimen collected after days' abstinence, Collected inside / outside the lab (May have medicolegalimportence whenever necessary), Received after collection, Examined after collection (Should be 30 mins after, collection. Before this time, the semen may remain coagulated, and hence, not properly mixed)

IMPRESSION:

---

References

WHO Manual for processing and examination of semen samples

## *3.* Urine Analysis

### 1. 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.
- Clean-Catch Prior to collection, clean the external genitalia with a mild antiseptic solution and dry. Allow the initial portion of the urine stream to escape, 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.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**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 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.

## Urine collection containers

**Urine Collection Containers** -leak-resistant cups

**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 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 not possible. A urine preservative of 6N HCI is available that allow urine to be kept at room temperature.

.

### 2. 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.
- Measure the pH of the urine on receipt of 24 hour sample in lab; it should be between 1 and 3. If the pH is high, few drops of 6N HCL can be added to the specimen to bring the pH between the desired range.

**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-resistent.
- Use containers that are made of break-resistant plastic, which is safer than glass.
- Specimen containers should not be reused.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Catheterized Catheterized specimens should not be routinely obtained for urinalysis although they may be submitted

**3. 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 differentcontainer. 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. Underfilling or overfilling containers with preservatives may affect specimen-to-additive ratios.

**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. 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.

**Procedure**

A qualitative chemical analysis of the urine is performed by using aUristix or multi-parameter test strip that measure pH, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, blood, leukocyte esterase, and specific gravity. The test strips are dipped in the urine and read visually according to the color comparison chart printed on the side of the container at prescribed time intervals.

Automated Urine Analysers can be used for this.

**Microscopic Examination**

1. Use fresh well-mixed urine collected by clean-catch method into a sterile container.

2. The specimen should be unpreserved. Pour five to ten milliliters of urine into a test tube.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Conical bottom test tubes are preferred because they allow for better pellet formation. Please the tube in the centrifuge and balance with a second tube filled with water (oranother urine specimen) of equal volume. Spin the urine for about five minutes. Remove the tube and decant the supernatant into the sink. Resuspend the sediment in the residual urine that clings to the bottom of the tube by tapping the tube against a hard surface several times. Place a drop of the resusupended sediment on a glass microscope slide using a pipette or by holding the tube upside down and carefully tapping it the slide until one drop falls onto the slide. Place a coverslip over the drop and place under the microscope.

3. Place the slide under the microscope with the low power objective (10X lens) and observe the sediment for the presence of casts. Observe at least ten (10) fields and take the average number of casts seen for each type.

4. Switch to high power objective (40X lens) to observe the sediment for the presence of epithelial cells, WBCs, RBCs, bacteria, crystals, yeast, trichomonas, mucus, and spermatozoa.

5. Scan approximately 8-10 fields and take the average per field for each type of sediment.

6. Urine samples are retained at 2-10°C for 24 hours in the Hematology refrigerator.

**The following urine samples are not satisfactory for testing:**

- Specimens received over two hours after collection.
- Mislabeled samples
- Improperly collected samples. For example, urine samples with preservatives, specimens collected in non-sterile containers, or specimens collected in containers with soap or detergent residues will not be accepted.
- QNS (Quantity Not Sufficient)
- In the event that an unacceptable sample is received, another sample must be requested.

**QUALITY CONTROL: (in urine Analyser)**

**Control Materials and Procedural Notes:**

A *Run* in urinalysis is defined as all patients testing performed in a 24-hour period. Quality Control mustbe performed under the following conditions.

- Every morning prior to testing patient specimens.
- Whenever test results appear questionable.

  - Normal and Abnormal Control will be performed at least once every *run*



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- If all results are within expected ranges, proceed with patient testing.

- If QC results are outside of expected ranges for controls:

- Re-examine the specimen using a different field of view.
- Adjust the coarse/fine focus as needed to improve the view and repeat testing.
- Check for signs of contamination in the controls.
- Repeat the procedure with freshly reconstituted controls.
- If the results are still out of range after performing the above steps, notify the next higher supervisor immediately. Corrective action must be taken and QC must be in range before patient testing can be performed.
- Record ***all*** QC values (both in and out of range) and corrective actions on the Quality Control Worksheets.

Lot Verification

All new quality control lots or separate shipments of current lots will be tested against the current lots for performance verification before performing patient testing. Results are considered acceptable if they are within their lot number quality control limits

**Backup tests and/or microscopic examination must be performed on urine that tests positive for the following:**

| **Positive Result** | **Backup Test** | **Microscopic Exam** |
|---|---|---|
| Blood | N/A | Yes |
| Nitrite | N/A | Yes |
| Ketones | Acetest | No |
| Glucose | Clinitest | No |
| Bilirubin | Ictotest | No |
| Protein | 3% Sulfosalicylic Acid | Yes |
| Leukocyte Esterase | N/A | Yes |

**Confirmatory tests are not required for TRACE results on PROTEIN or KETONES**.

**Microscopic examinations are required on the following specimens regardless of the reagent strip results:**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Urine with cloudy or hazy appearance.
- Microscopic examination requested by the patient's physician.
- Urine specimens received from Pediatric Clinic.
- Catheterized and Suprapubic aspiration samples.

**Expected / critical results & reportable range:**

1. The following **bolded analytes** require a result entry during the microscopic results even if they are not seen during the microscopic exam. Report "none" or 0-1 or 0-2 as indicated. All other analytes are optional entry if seen during the microscopic exam.

| **Analyte** | **Expected results for: ALL AGES** | **Reportable Range:** |
|---|---|---|
| **HYALINE CASTS** | 0-1 | 0-1, 1-3, 3-5, 5-10, 10-25, 25-50, or greater than (>) 50/ LPF. Quantify each cast type separately |
| Granular Cast | None | |
| Cellular Cast (RBC, WBC, etc) | None | |
| Waxy Cast | None | |
| **EPITHELIAL CELLS** | 0-2 | 0-2, 2-5, 5-10, 10-25, 25-50, or greater than (>) 50/ HPF |
| **LEUKOCYTES (WHITE BLOOD CELLS)** | 0-2 | 0-2, 2-5, 5-10, 10-25, 25-50, or greater than (>) 50/ HPF |
| **RED BLOOD CELLS** | 0-2 | 0-2, 2-5, 5-10, 10-25, 25-50, or greater than (>) 50/ HPF |
| **BACTERIA** | None - Few | None, Few, Trace, Moderate or Many |
| **MUCUS** | None - Light | Light, Moderate or Heavy |
| Crystals | None | Few, Moderate or Many for each crystal type. |
| Spermatozoa | Males only: Few | Few, Moderate, or Many. |
| Yeast | None | Light, Moderate or Many. Report any budding yeast or hyphea seen using comments as noted below. |
| Trichomonas | None | Few, Moderate or Many. May only be reported if motile. |

2. If budding yeast or hyphea are present, add the following canned comments:

[Budding] - ***Budding yeast present***.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

[Hyphea] - ***Hyphae present***.

3. If a urine dipstick is performed, compare the results obtained on the microscopic with the multi-parameter reagent strip with the following facts in mind:

Urine with RBCs seen on the microscopic exam should have a positive occult blood on the reagent strip.

Urine with casts should have elevated specific gravity and positive protein.

Urine with crystals should have elevated specific gravity.

Urine with positive nitrite should have bacteria on the microscopic.

**5. Critical values:**

When the following values are encountered after a urinalysis testing, immediately notifythe physician or senior ward/clinic nurse of the values encountered.

| **Analyte** | **Critical Results: All ages unless otherwise noted** |
|---|---|
| Waxy Casts | Any |
| Red Blood Cell Cast | Any |
| Cystine Crystals | Any |
| Tyrosine Crystals | Any |
| Leucine Crystals | Any |

Critical values must be reported in accordance with the laboratory critical value policy. .

**Test for Bile Pigment**

**Fouchets Test-**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

To 5 ml of urine in a test tube add 5 ml of 10% barium chloride.

Shake well.

Filter it off.

Let the filter paper dry.

When dry, add 1-2 drops of Fouchet's reagent to the dried precipitate.

A Green colour indicates bilirubinuria.

**Procedure to detect bile salts in urine**

**Hays' Sulphur Test-**

Bile salts reduce the surface tension of liquids.

On this principle HAY's test is done.

Sulphur powder is sprinkled over the surface of urine and it sinks if bile salts are present.

Sulphur will remain over the surface when bile salts are absent.

**Test for Ketone Bodies in Urine**

**Rothera Test**

Aim

To detect whether acetone or ketone bodies are present in the given sample of urine.

Apparatus

Test tube, pipette

Procedure

Saturate about 5ml of urine with ammonium sulphate. Add few crystals of sodium nitropruside and shake well. Add liquor ammonia through the sides of the test tube slowly at an angle of 45 degrees, formation of a purple ring at the junction indicate a +ve test. The ketone bodies that may be present are acetone, acetoacetic acid and —hydroxyburyric acid.

**Test for Proteins in urine**

**Sulphosalicylic Acid Test**

**Aim**

To detect whether proteins are present in the given sample of urine.

To 1 ml of Urine add 3 drops of 20% sulphosalicylic acid.

Absence of cloudiness means absence of proteins.

If the turbidity persists after boiling, it is due to protein. If the cloudiness vanishes on heating and reappears on cooling, it is due to Bence Jones Proteins.

## References:


1. Stransinger, Susan K., Urinalysis and Body Fluids, Third Edition, F.A. Davis Book Publisher, 1994, Pages 1 to 10 and 51 to 74.

2. Haber, Meryl H., Urinary Sediment: A Textbook Atlas, American Society of Clinical Pathologist Book Publisher, 1994.

3 Multistix 10 with SG Package Insert, Bayer Corporation; Diagnostics Division, 1999.

4 NCCLS GP-16A2, Volume 21, No. 19, Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline-Second Edition, p. 4-21.


## 3. Stool examination

**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.
- 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. 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
- 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

- 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

**Physical Examination of stool**

- Quantity
- Form
- Consistency
- Colour
- Size and shape-formed
- Gross Blood –present/absent
- Mucus-present/absent
- Pus-present/absent
- Parasites-present/absent
- Pus-present/absent
- Parasites-present/absent
- Fat-present/absent
- Undigested elements-present/absent
- Stool pH- To determine the pH of stool, simply touch the pH paper to a moist sample

**Microscopic Examination of stool**

Apparatus Required

1. Glass slides
2. Over slip 22x22 mm
3. Pasteur Pipette
4. Applicators
5. Lugols Iodine Solution
6. Normal Saline
7. Microscope

Procedure

1. Normal saline is used for routine examination of stool specimens. Use fresh uninfected saline. Place a small piece of stool on a slide and mix with saline until smooth. Cover with the cover slip.
2. Iodine is used to examine the nuclear structure of cysts; the preparation is made the same way as in saline. Saline and Iodine preparation can be made on the same slide using two different cover slips.
3. If the specimen contains mucus, examine preferably without saline
4. Examine under 10x and 40x objectives, with a reduced condenser aperture
5. Report the presence of
    a. large number of pus cells,
    b. muscle fibres, Red blood cells,
    c. free living amoebae, flagellates or ciliates,
    d. eggs and larvae,
    e. cysts
    f. yeast cells
6. Report parasitis amoebae, flagellates, ciliates, eggs larvae and cysts as the number seen in theentire preparation

a. scanty 1-3
b. few 1+ (4-10)
c. Moderate Number 2+ (10-20)
d. Many 3 + (20-40)
e. Very many 4+ (>40)

**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

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



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Radishes

Red meat (especially meat that is cooked rare)

Turnips

Vitamin C-enriched foods or beverages

Procedure: Follow the procedure as per the kit insert.

## References


- Cook, JH, and M Pezzlo (1992). Specimen receipt and accessioning. Section 1. Aerobic bacteriology, 1.2.1-4. In HD Isenberg (ed) Clinical Microbiology Procedures Handbook. American Society for Microbiology, Washington DC
- Miller, J Michael (1999) A Guide To Specimen Management in Clinical Microbiology, American Society for Microbiology, Washington DC
- Fischbach FT, Dunning MB III, eds. (2009). Manual of Laboratory and Diagnostic Tests, 8th ed. Philadelphia: Lippincott Williams and Wilkins.
- Parasitology (Protozoology and Helminthology) by K D Chatterjee 2009




Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 3. HEMATOLOGY

**Purpose:** Handling of Blood Samples for hematology related tests and procedures

**Scope**: To Perform Haematology Tests on the Blood Samples

**Responsibility:** Lab Incharge/ Pathologist

**Procedure**: as under

| Sr. No. | Activity/ Description | Responsibility | Ref. Doc. Record |
|---|---|---|---|
| | **3.4.1-3.4.6 Refer To Collection Manual** | Technical Staff posted at Collection Counter | |
| | **Instructions for repeat testing**<br><br>Under following conditions repeat tests are performed:<br><br>9. When the results are beyond thelinearity limit, sample is tested in appropriate dilution / concentration.<br>10. Highly abnormal results are retested for confirmation.<br>11. When the results do not correlate with other results.<br>12. On request of clinicians / patient.<br>13. Disparity between cell counter and microscopic findings.<br>14. Improper staining<br>15. Abnormal result for cross check | Technical Staff Counterchecked by Lab Incharge | |
| | **Process Efficiency Criteria OPD and IPD (Minimum Desired)**<br>• Repeat Testing <1%<br>• Staining Quality<br>• Any Discrepancy Between cell counter findings and peripheral smear reporting<br>• Tailing artefacts /any other artefacts in the smear preparation<br>(each hospital to set their own Criteria) | Senior Most Technical Staff Monitored by the Lab Incharge | Should be documented in the quality control register/ |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | • Compliance with the Turn Around Time set (as under)<br>• Timely reporting and documentation of Critical Reports as per the criteria | Senior Technician to coordinate with the dispatch staff and monitored by the Lab Incharge | |

| | | | |
|---|---|---|---|
| | **3.4.9 Critical reference value and procedure**:<br>**Refer to Annexure**<br><br>Critical values are made as per clinicalcritical levels of different sections of the lab. **All the critical values are informed both telephonically to the concerned doctor and by critical alert movement register maintained by lab.** | Technician on duty and Doctors on duty | A Critical Value movement register* is maintained by the lab to the concerned departments.<br>(*as per the individual Hospital defined criteria) |
| | **3.4.10 Release of report**:<br>All the lab reports are dispatched after final reporting by the lab doctor. The turn around time of the lab reports are<br>• CBC on same day.<br>• Emergency reports released on same day within 2 hours . | Technical Staff on duty | Dispatch register is maintained.<br>Emergency sample register is maintained. |
| | **3.4.11Internal Quality control system**<br>It is a regular process carried out in the lab to standardize the lab system of all sections by-<br>1. By running internal quality control (commercially available) routinely, at the beginning of processing of all the routine samples.<br>2. Repeat runs on the random samples<br>3. Matching the machine report with microscopic report.<br>4. Running same sample on two machines if available | Senior Technician supervised by Department Incharge | Internal quality control registers are maintained. |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **3.4.12External quality control system**<br>EQAS is maintained by participating in EQAS (External quality assessment system) conducted by recognized institutes like CMC Vellore, AIIMS etc. External quality samples are sent by the institute on quarterly basis and reports of the participating hospitals for the same are sent accordingly within given time which is cross checked and evaluated for quality control.<br>Reports of assessment are sent to the respective hospital. Annually a conduct certificate is issued. | Senior Technician supervised by Department Incharge | External quality control register and file is maintained. |
| | **3.4.13 Calibrations of equipment**<br>All the lab equipments are to be calibrated periodically by the company engineer.(six monthly) | Senior Technician supervised by Department Incharge | A log book and files are to be maintained. |
| | **3.4.14 Validation of reagents, stains, kits** etc:<br>• Random re -sampling<br>• By checking the Lot, batch no, and expiry date.<br>• Cross checking of the slides for microscopic findings.<br>• For validation of stains, control slides are checked. | Senior Technician supervised by Department Incharge | Quality Control Register to be maintained. |
| | **3.4.15 System of resolution of complaints** and feedbacks from stakeholder.<br>Any lab related complaint and feedbacks are to be resolved by the department incharge and patient satisfaction should be the priority. | Senior Technician supervised by Department Incharge | Complaints and feedback register is maintained |
| | **3.4.16 Referral laboratories** (as per the State Government Policy) | | |
| | 3.**4.17 Storage, retaining and retrieval** of -.<br>Priority should be given to timely dispatch of the reports as per the Turn Around Time.<br>All uncollected/unclaimed Lab reports are to be stored for 1 week only<br><br>**Preferably HIMS system should be in place**<br><br>*Storage of samples*<br>Primary and examination samples<br>• EDTA Blood samples 24 hours. | Senior Technician supervised by Department Incharge<br>To be communicated to the concerned OPD/IPD incharge | Dispatch registers to be maintained<br>Each hospital to set their own dispatch procedure |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | | | |
| | **3.4.18 Control of documents**-<br>• Random checking of the documents<br>• Checking the page numbering<br>• Signing of the documents<br>• Removal of obsolete documents and<br>• Any modification to be done | Senior Technician supervised by Department Incharge | Register maintained. |
| | **3.4.19 Procedure for preventive and break down maintenance**:<br>• Separate system for collection and handling of sample.<br>• Urgent / instant sampling preferred.<br>• Urgent reporting and dispatching.<br>• Separate register is maintained.<br>• Consultation with treating doctor if required.<br>• Regular information to the Nodal officer dealing epidemic breakdown, through documentation. | Senior Technician supervised by Department Incharge | Separate register for epidemic or breakdown is to be maintained. |
| | **3.4.20 Procedure for internal audit**<br>• All lab stock registers with page numbering are checked time to time.<br>• Lots, batch no of the kits, Date of Expiry and reagents are checked time to time.<br>• FIFO for reagents (first in first out) system is followed. *as per the available stock<br>• All the existing equipments/ machine/ non consumables are checked time to time and tally with the register. | Lab store incharge and Department incharge. | Stock register is maintained. |
| | **3.4.21 Procedures for purchase of external services and supplies**:<br><br>As per the hospital procedure and policy. | Purchase Section | |

**Annexures**

**1. Screening of blood groups**

Qualitative tests for ABO grouping, with antisera

**Principle**

The procedures used with the antisera are based on the principle of agglutination. Normal human red cells possessing antigen, will clump in the presence of corresponding antibody**.**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Method** Slide method

**Requirements**

1. Glass slides/Tiles
2. Pasteur pipette/Autopipettes
3. Applicator
4. Centrifuge machine

**Reagents**

1. Anti –A sera
2. Anti – B sera
3. Normalsaline

**Specimen**

EDTA blood

**Procedure**

1. Prepare a 10% suspension of red cells in normal saline.
   i.e. Mix 5 drops of RBC with 2ml of normal saline. Centrifuge at 1500rpm for 1-2 minutes. Discard supernatant. Add 2ml of normal saline to the sedimented red cells. Mix well. This gives a 10% suspension of red cells.
2. On one half of a glass slides, place 1 drop of Anti- A blood grouping serum.
3. On the other half of the slide, place 1 drop of Anti-B blood grouping serum.
4. Using a Pasteur pipette add 1 drop of the cell suspension to each hail of the slide.
5. With separate applicator sticks, mix each cell- serum mixture well.
6. Tilt the slide back and forth and observe for agglutination.

**Interpretation**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| Reaction<br>Anti- A | Anti-B | Interpretation<br>Group |
|---|---|---|
| + | 0 | A |
| 0 | + | B |
| + | + | AB |
| 0 | 0 | 0 |

**0: No agglutination**

**+: Agglutination**

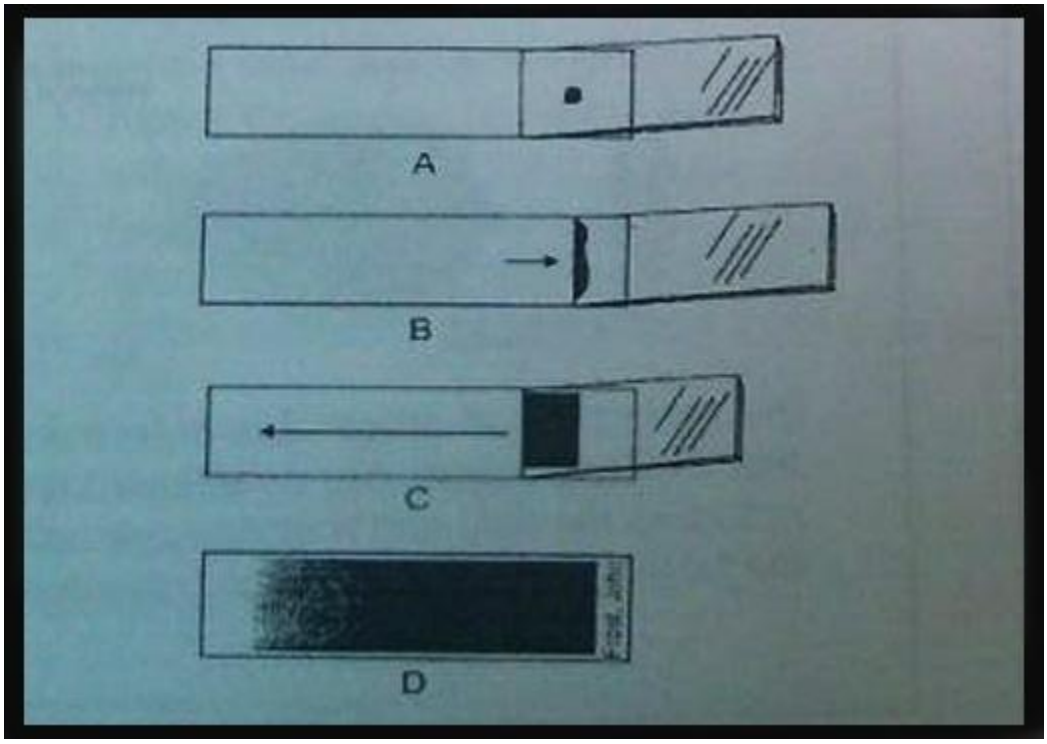
***Note:*** **Blood obtained by finger prick may be tested directly by the slide method. To avoid clotting the collected blood should be mixed quickly with the antisera and a note should be clearly mentioned on the report that it is a screening method**

**2. Procedure for peripheral blood smear preparation**

After receiving the blood sample in the work station in the lab, sample is checked and matched with the requisition form. Haemogram sample is run in the analyser and peripheral blood smear is prepared as per request.

**Steps in PBS preparation**

- Place a 1"x 3" glass microscope slide with a frosted end on a flat surface (usually the counter top of a laboratory bench).
- Attach a label on the slide or write the patient's name, specimen identification number, and date of preparation on the frosted surface.
- Place a 2 – 3 mm drop of blood approximately 1 cm from the frosted end of the glass slide.
- Hold the slide between the thumb and forefinger of one hand at the end farthest from the frosted end.
- Grasp a second slide (" spreader slide") between the thumb and forefinger of the other hand at the frosted end
- Place the edge of the spreader slide on the lower slide in front of the drop of blood.




**Characteristics of well made smear**

- Tongue shaped: smear is thick at the frosted end and becomes progressively thinner toward the opposite end.
- The “zone of morphology” (area of optional thickness for light microscopic examination) should be at least 2 cm in length.
- The smear should occupy the central area of the slide and the margin – free at the edges.
- Blood film should be smooth and free of serration.
- Unstained smear should be transparent so that newsprint is visible through it.
- Make at least two smears.

An ideal smear should have at least 2 cm zone of morphology windows in smear occurs due to wing a greasy slide.


**Drying of Blood Film**

- Air-drying without forced air circulation is sufficient.
- In humid conditions, forced air-drying is recommended.
- Slow dying causes cells to contract, whereas water in excess can cause gross morphological artefacts, such as decreased “crispness” of cellular appearance & the development of artificial vacuoles.

**Fixation**

- Fixation preserves the morphology of the cells.
- Optimal results are obtained by fixing and staining immediately after the blood film is completely air-dried.
- If slides cannot be stained immediately, fixation in methanol is necessary within 4 hours, but preferably 1 hour after air – drying. (for Giemsa Staining)

**Common causes of a poor blood smear**

1. Dirty / greasy slide.
2. Drop of blood too large or too small.
3. Edge of the spreader was not smooth.
4. Spreader slide pushed across the slide in a jerky manner.
5. Failure to keep the entire edge of the spreader slide against the slide while making the smear.
6. Failure to keep the spreader slide at a 30” angle with the slide.
7. Improper drying.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

8. Improper pH of the stain/ buffer.
9. Old stain.
10. Staining surface is not leveled.

**3. Giemsa Staining**

**Purpose**: To examine a smear

**Reagents**: Methanol, giemsa stain (as per the Availability of readymade/lab Prepared Reagent)

**Specimen**: Blood

**Equipment**: a) Glass slides b) Pipettes

**Procedure:**

1. Make a smear in a clean glass slide and air dry.
2. Fix in ten dips in Methanol and air dry.
3. Covers slides with 1:10 diluted giemsa stain with Buffer
4. Wait for 10 min-15 mins
5. Wash carefully with running tap water and final dip in the Buffer
6. Dry and observe under microscope

**4. Leishman's Staining**

**Purpose**: To examine a peripheral smear
**Reagents**: a) Leishman's Stain b) Distilled Water
**Specimen**: Blood.
**Equipment**: a) Glass slides b) Pipettes c) Staining rack
**Procedure**:

1. Make a smear in a clean glass slide and air dry .
2. Cover the smear with Leishman's Stain (Readymade/ Lab prepared)
3. Wait for 2 min.
4. Add double volume of distilled / buffer water.
5. Wait for 8 – 10 min.
6. Wash in running tap water.
7. Air dry
8. Observe under oil immersion.

**Interpretation:** As per microscopic findings.

Quality Control: Compared with internal quality control slides



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Interpretation:** As per microscopic findings

Quality Control: Compared with internal quality control slides

Majority of the Hematology investigations complete blood cell counts are performed on 3 part/5 parts automated analyzer . Procedure is followed as per analyser manual.

**5. Automated hematology analyser**
Used for haemotology parameters.

1. Check the machine for reagents, sufficient quantity of the reagent should be present.
2. In order to avoid any error the quantity of the reagent should be adequate.
3. After unpacking the reagent, care should be taken to prevent entry of dust, dirt bacteria etc.
4. Inspect that there are no broken tubing and power cord is plugged in the outlet.
5. Waste tank should be checked daily.
6. Check for the printer paper.

Use reagents as per the instructions in reagent insert and follow the instrument Manual

**Procedure in Analysis Mode**

Whole Blood Mode:

Sample collection and preparation

1. The sample quantity should be sufficient as per the instrument Manual
2. The technician should look for the clots in the samples and document and ask for repeat sample.

Sample Analysis

1. Mix the sample on the mixer.
2. Care should be taken while removing the tube cover to prevent blood scatter wherever the cap piercing facility is not available in the instrument.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Responsibility**:

All the Hematological parameters generated by the analyzer are checked by the technician incharge and any abnormal value or values in the critical range is brought to notice of the pathologist incharge and values are re-checked before dispatching the final report. This is done by blood smear examination and manual methods if needed by the pathologist.

## Maintenance

**Daily:**

1. Run QC daily.
2. E-Z cleaning.
3. System shut down.

**Biweekly:**

1. ZAP aperture.
2. Flush aperture.
3. Entire system cleaning.

### 6. Determination of erythrocyte sedimentation rate

## Methods

**Westergren's method**

Normal range

- Male 0-15 mm after 1$^{st}$ hour
- Female 0-20 mm after 1$^{st}$ hour

**Specimens**

1. Fasting EDTA blood sample

2. Blood in the tube with of 3.8% sodium citrate in the ratio of 1:9 ( sodiumcitrate: Blood)

**Requirements**

- Westergren's ESR tube
- Stand for holding the tube
- Timer and watch

**Procedure**

1. Fill the Westergren tube exactly upto zero mark by means of a rubber bulb.
2. Place the tube upright in the stand. It should s fit evenly into the groove of the stand.
3. Note the time. Allow the tube to stand for exactly one hour.
4. Exactly after one hour, note the level to which the red cell column has fallen.
5. Report the result in terms of mm/after 1st hour.

**Wintrobe method**

Normal range-

Male 0-9mm/after 1st hour

Female 0-20mm/after 1st hour

**Specimen**

Fresh fasting EDTA blood sample

REQUIREMENTS

1. Wintrobe tube
2. Wintrobe tube stand
3. Pasteur pipette
4. Timer and watch

**Procedure**

1. Mix the blood carefully.

2. Fill the wintrobe tube upto the zero mark by using Pasteur pipette.
3. Place the tube in exact vertical position in the stand. Set the timer for one hour.
4. At the end of the one hour note the level of erythrocyte column in terms of mm after 1st hour

****Use Automated ESR system if available as per the instrument Manual**

**7. Determination of reticulocyte count**

The number of reticulocyte count in the peripheral blood is a reflection of red cell forming activity i.e. Erythropoietic activity of the bone marrow. Increase in their number indicatesincrease activity of the marrow. This is known as reticulocytosis. Low count or absence of reticulocytes indicates bone marrow suppression.

**Normal range**

Adult-0.2 to 2%

Infant- 2-6%

**Specimen**

EDTA or HEPARINIZED BLOOD. Perform the test within 2-3 hours of blood collection.

**Principle**

Supravital staining method is used for reticulocyte count. Blood is mixed with the stain and the stain enters the cells in living condition. The RNA in the cells is precipitated by staining as dark blue reticulum. A relative count is taken against the number of red blood cells and expressed as a percentage of red cells.

**Requirements**

1. Glass slides
2. Test tubes(15x125mm)

3. Pasteur pipette
4. Test tube rack
5. Microscope
6. Reagents: staining solutions
    - Brilliant cresyl blue
    - New methylene blue

**Procedure**

1. Filter a small amount of the stain about 5ml.
2. Add two drops of blood and two drops of the stain by using separate Pasteur pipette and mix thoroughly.
3. Incubate at 37'c for 30 minutes.
4. Prepare a thin smear of the stained blood specimen by using a spreader slide. Air dry the smear. Change to the oil immersion objective.
5. First examine the smear under the low power objective and locate a portion of the smear where the red cells are evenly distributed.
6. Reticulocyte are identified by fine, deep violet filament and granules arranged in a network. Red cells are stained pale blue.
7. Count reticulocytes and red cells in about 15 fields.
8. Calculations

   Reticulocyte percentage

   = $\frac{\text{Number of reticulocytes counted x 100}}{\text{Number of red cell counted}}$ = %age

## 8. Critical Alert Values

All the critical values are informed both telephonically to the concerned doctor and by critical alert movement register maintained by lab.

**1)** Critical values have been defined on the input of the clinicians of the hospital and is attached as below.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**2)** On testing the samples if any values are found to be in the critical zone the technician on duty immediately informs the clinician /sister in charge so that the same is communicated to the attending clinician.

- Activated Partial Thromboplastin Time, Plasma - ≥ 150 sec
- Fibrinogen ≤ 60 - mg/dL
- Hemoglobin 0-7 weeks ≤ 6.0 or ≥ 24.0 g/dL
- Hemoglobin>7 weeks ≤ 6.0 or ≥ 20.0 g/dL
- INR (International Normalizing Ratio) - ≥ 5.0
- Leukocytes - ≥ 100.0 x10(9)/L
- Absolute Neutrophil Count ≤ 0.5 - x10(9)/L
- Neutrophils ≤ 0.5 - x10(9)/L
- Platelets, Blood ≤ 40 or ≥ 1000 x10(9)/L
- CSF White Blood Cell Count ≥ 100.0 Cells/mcL
- Alert Blasts >2% blasts with anemia, neutropenia, or thrombocytopenia
- Total WBC < 0.5
- Hematocrit - All Ages < 18% ; >90 days old > 60%
- Positive blood smears for Falciparum Malaria or positive Malaria serology for Falciparum
- Positive sickle cell on blood smear in previously unknown patient

## Registers

1. Lab Register
2. Individual Bench register eg ESR
3. Quality Control Register
4. EQAS register
5. Complaint register
6. Critical Value Register
7. Log Books
8. Indent register/Stock Register



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Following table describes the Responsibility of Document Control of the listed documents:**

| Name of Document | Document Control | Person Responsible | | |
|---|---|---|---|---|
| | | **Preparation** | **Amendment** | **Approval & Review** |
| Quality Manual | Quality Manual has a Title, Prepared andissued by & date,Approved by & date, Effective date, Version number, copy number & total number of pagesand reviewed on date & signature. | Section I/C | Section I/C | HOD |
| Quality system Procedures | Quality System Procedure have a title, document number, Prepared and issued by & date, Approved by & date, Effective date, version number, copy numberand total number of pages and reviewed on date & signature. | Section I/C | Section I/C | HOD |
| Standard Operating Procedures | Standard Operating Procedures have details such as a title, document number, prepared and issued by & date, approved by & date, Effective date, version number, copy number and total number of pages and reviewed on date and signature. | Section I/C | Section I/C | HOD/Pathologist Review – Annual |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## a. ADD ON TEST – ON VERBAL REQUEST

**Purpose and scope**

The purpose of having the policy of add on test on verbal request is to assist the treating doctor to evaluate the patient condition more critically and in cases where additional test could may provide additional inputs.

**Responsibility**

Technician / Section Head

## b. BLIND TESTING PROGRAME

**Purpose and scope**

The blind testing programme shall be carried out for quality assurance. This will be done more frequently for the parameters for which there is no EQAS programme available. This increases the confidence in reporting results.

**Responsibility**

Technician / Section head.

**Procedure**

1. On receiving the samples in the Lab, the section head will randomly select few samples. The section head will ascertain that there is sufficient sample volume to be split in to two vials. The section head will than divide the sample in two vials. The section head will give different identification to the split sample and will ask the technician to perform the test on both vials. The technician will then submit the results of both vials to the section head.

2. The section head will then match the results received on both vials, calculate the measurement of uncertainty (MU).
3. In case of outlier, the section head will initiate necessary corrective action as per laid procedure and document it.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## Reference Documents

1. Practical haematology –Sir John v. Dacie and s.m. lewis
2. Textbook of medical laboratory technology - Praful b. Godkar and Darshan b. Godkar



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 4. BIOCHEMISTRY

**4.1. Purpose**: Handling of Blood Samples for biochemistry and immunochemistry

**4.2.Scope**: To Perform biochemistry and immunochemistry on the Blood Samples

**4.3.Responsibility**: Lab Incharge/ Biochemistry

**Procedure**: as under

| Sr. No. | Activity/ Description | Responsibility | Ref. Doc. Record |
|---|---|---|---|
| | **4.4.1-4.4.6 Refer To Collection Manual** | Technical Staff posted at Collection Counter | |
| | **4.4.7 Instructions for repeat testing** | Technical Staff Counterchecked by Lab Incharge | |
| | Under following conditions repeat tests areperformed:<br>16. When the results are beyond the linearity limit, sample is tested in appropriate dilution / concentration.<br>17. Highly abnormal results are retested for confirmation.<br>18. When the results do not correlate with other results.<br>19. On request of clinicians / patient.<br>20. Abnormal result for cross check | | |
| | **4.4.8 Process Efficiency Criteria OPD and IPD (Minimum Desired)**<br>• Repeat Testing <1%<br>• Compliance with the Turn Around Time set (as under)<br>• Timely reporting and documentation of Critical Reports as per the criteria | Senior Most Technical Staff Monitored by the Lab Incharge<br>Senior Technician To coordinate with the dispatch staff and monitored by the | Should be documented in the quality control register/ |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | | Lab Incharge | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **4.4.9 Biological reference intervals.** | Lab incharge and if the consultant believes that the particular interval is no longer useful for the reference population, then the complete investigation is undertaken and the corrective action is initiated | Reference to be taken from kit inserts and text books, |
| | **4.4.10 Critical reference value and procedure:**<br><br>Biochemistry. **Refer to Annexure**<br>Critical values are made as per clinical critical levels of different sections of the lab. All the critical values are informed both telephonically to the concerned doctor and by critical alert movement register maintained by lab. | Technician on duty and Doctors on duty | A Critical Value movement register* is maintained by the lab to the concerned departments.<br>(*as per the individual Hospital defined criteria) |
| | **4.4.11 Release of report:**<br>All the lab reports are dispatched after final reporting by the lab doctor. The turn around time of the lab reports are<br>• Biochemistry on same day.<br>• Immunochemistry third day<br>• Emergency reports released on same day within 2 hours. | Technical Staff on duty | Dispatch register is maintained.<br>Emergency sample register is maintained. |
| | **4.4.12 Internal Quality control system**<br>It is a regular process carried out in the lab tostandardise the lab system of all sections by-<br>1. By running internal quality control (commercially available) routinely, at the beginning of processing of all the routine samples.<br>2. Repeat runs on the random samples<br>3.Interequipmentsample being run on two machines if available | Senior Technician supervised by Department Incharge | Internal quality control registers are maintained. |
| | **4.4.13 External quality control (EQC)system**<br>EQC is maintained by participating in Quality Control programme conducted by recognised institutes like CMC Vellore, or various reputed companies etc. External quality samples are sent by on quarterly basis or yearly | Senior Technician supervised by Department Incharge | External quality control register and file is maintained. |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | as per the policy and reports of the participating hospitals for the same are sent accordingly within given time which is cross checked and evaluated for quality control .<br>Reports of assessment are sent to the respective hospital.<br>Annually a conduct certificate is issued. | | |
| | **4.4.14 Calibrations of equipment**<br>All the lab equipments are to be calibrated periodically by the company engineer.(six monthly) | Senior Technician supervised by Department Incharge | A log book and files are to be maintained. |
| | **4.4.15 Validation of reagents, kits etc:**<br>• Random re -sampling<br>• By running By checking the Lot, batch no, and expiry date.<br>• samples for linearity , accuracy<br>• By processing patient's sample on old kit and new kit | Senior Technician supervised by Department Incharge | Quality Control Register for IQC and EQC to be maintained. |
| | **4.4.16 System of resolution of complaints and feedbacks from stakeholder.**<br>Any lab related complaint and feedbacks are to be resolved by the department incharge and patient satisfaction should be the priority. | Senior Technician supervised by Department Incharge | Complaints and feedback register is maintained |
| | **4.4.17 Referral laboratories** (as per the State Government Policy) | | |
| | **4.4.18 Storage, retaining and retrieval of lab records.***<br>Priority should be given to timely dispatch of the reports as per the Turn Around Time.<br>All uncollected/unclaimed Lab reports are to be stored for 1 week only<br>*each hospital may have their own policy<br>Calibration record to be kept for one year<br>Quality control record to be kept for one year<br><br>**Preferably HIMS system should be in place**<br><br>*Storage of samples*<br>Primary and examination samples<br>• Blood samples for routine biochemistry andimmunochemistry 48 hours (2-8C). | Senior Technician supervised by Department Incharge<br>To be communicated to the concerned OPD/IPD incharge | Dispatch registers to be maintained<br>Each hospital to set their own dispatch procedure |
| | **4.4.19 Control of documents-**<br>• Random checking of the documents<br>• Checking the page numbering<br>• Signing of the documents<br>• Removal of obsolete documents and<br>• Any modification to be done | Senior Technician supervised by Department Incharge | Register maintained. |
| | **4.4.20 Procedure for preventive and break down maintenance:** | Senior Technician supervised by | Log book,/Separate service file to be maintained. |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | • The maintenance protocol is yearly for preventive maintenance/as per procurement protocols<br>• In case of breakdown the maintenance is to be done as an when required | Department Incharge | |
| | **4.4.21 Procedure for internal audit**<br>• All lab stock registers with page numbering are checked time to time.<br>• Lots, batch no of the kits, Date of Expiry and reagents are checked time to time.<br>• FIFO for reagents (first in first out) system is followed.<br>• All the existing equipments/ machine/ non consumables are checked time to time and tally with the register.<br>• Status for calibrations and maintenance to be checked | Lab store incharge and Deparment incharge. | Stock register is maintained.<br>Log book of Equipments is checked |
| | **4.4.22 Procedures for purchase of external services and supplies:**<br><br>As per the hospital procedure and policy. | Purchase Section | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Following table describes the Responsibility of Document Control of the listed documents:**

| **Name of Document** | **Document Control** | **Person Responsible** | | |
|---|---|---|---|---|
| | | **Preparation** | **Amendment** | **Approval & Review** |
| Quality Manual | Quality Manual has a Title, Prepared and issued by & date, Approved by & date, Effective date, Version number, copy number & total number of pages and reviewed on date & signature. | Section I/C | Section I/C | HOD |
| Quality system Procedures | Quality System Procedure have a title, Document number, Prepared and issued by & date, Approved by & date, Effective date, version number, copy number and total number of pages and reviewed on date & signature. | Section I/C | Section I/C | HOD |
| Standard Operating Procedures | Standard Operating Procedures have details such as a title, document number, prepared and issued by & date, approved by & date, Effective date, version number, copy number and total number of pages and reviewed on date and signature. | Section I/C | Section I/C | HOD/biochemist Review – Annual or as required |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## List of registers

1. Internal Quality control registers
2. External quality control register- Corrective action and preventive action
3. Calibration register
4. Log book
5. Panic value register
6. Phlebotomy quality register- SNR, QNS, Hemolysed sample
7. Cross check register
8. Departmental indent register
9. Reporting error register
10. Lot verification register
11. distillation plant water resistivity register

## Annexure

## 1. Critical values in biochemistry

All the critical values are informed both telephonically to the concerned doctor and by critical alert movement register maintained by lab.

| Clinical Biochemistry | Units | Low | High |
|---|---|---|---|
| Serum Calcium | mg/dL | ≤6.0 | ≥13.0 |
| Serum ionized Calcium | mg/dl | <3.2 | ≥6.2 |
| Serum Glucose | mg/dL | ≤40 | ≥450 |
| Serum Magnesium | mg/dL | ≤1.0 | ≥4.0 |
| Serum Sodium | mEq/L | ≤120 | ≥160 |
| Serum Potassium | mEq/L | ≤2.5 | ≥6.0 |
| Serum BUN | mg/dl | None | ≥80.0 |
| Serum Creatinine | mg/dl | None | ≥5.0 |
| Serum Bilirubin (total - newborn) | mg/dL | | ≥15 |

**Reference**: 1.Burtis C.A, Ashwood E.R., Bruns D.E. Textbook of clinical chemistry and molecular Diagnostics, 5th Edition

## 2. Corrective Action Plan

**Purpose and scope**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

In order to analyze and take immediate action for any outliers in IQC & / or EQAS results the root cause analysis is done and following corrective actions are immediately initiated

**Responsibility**

Technician/doctors

**Procedure**

Two level IQC is run before starting the samples.

a) If there is outlier in single level of control then this is taken as a warning sign.
b) If both the level of controls are beyond 2SD then the following is done.
c) Firstly fresh calibration is done
d) If still there is an outlier of the quality control sample then change the reagent
e) In case the error cannot be rectified, the service engineer from the company is called

immediately to rectify the fault and the work is either halted or done on stand equipment.

**SOP for centrifugation of samples**

Follow the steps below when preparing a serum specimen for submission.

**Purpose:**

Centrifuges are used to separate components of a mixture on the basis of particle size or density. The most common application in the clinical laboratory is the separation of blood into cells and either serum (from blood collected without an anticoagulant) or plasma (from blood collected with an anticoagulant such as EDTA). Each application requires a specific centrifugal force and a defined time period.

Although it is common to see centrifugation instructions specify the revolutions per minute (rpm) to be used, the only time this is valid is if the centrifuge and its rotor head radius are also listed. The more valid parameter is the relative centrifugal force (rcf).

**I. Personnel:**

These guidelines apply to anyone who prepares specimens for clinical testing or performs clinical tests on human specimens

**II. Guidelines:**

**Note:** If working with clot tubes (those collected without anticoagulant), do not begin to centrifuge blood specimens until adequate clotting has occurred. Clotting generally occurs within 20 to 60 minutes at 22 to 25 degrees centigrade without the aid of clotting activators

**IV. Procedure:**

1. Draw whole blood in an amount 2½ times the required volume of serum so that a sufficient amount of serum can be obtained.
2. Label the specimen appropriately.
3. Place the collection tube in the upright position in the rack, and allow the blood to clot at room temperature for no longer than 30-60 minutes. If clotting fails to occur within 60 minutes, notify the physician. Do not remove the tube stopper.
4. After allowing clot to form, insert the tube in the centrifuge, stopper end up. Operate the centrifuge for no more than 10 minutes at the speed recommended by the manufacturer. Prolonged centrifugation may cause hemolysis. When using a bench-top centrifuge, employ a balance tube of the same type containing an equivalent volume of water.

**5. Recommended Centrifuge Time and Relative Centrifugal Force (rcf)**

Time: 10 +/- 5 minutes RCF: 1000 – 1200 X g
(g = relative centrifugal force and is measured in multiples of the earth's gravitational field). Current laboratory practice appears to be "state of the art," i.e., separating serum or plasma fromcells is based on empirical observation. However, when using serum separating tubes, consult the manufacturer's literature for specific recommendations.

Guideline for calculation of RCF:
rcf = 1.118 X $10^{-5}$ X r X $n^2$ where r = rotating radius (cm), i.e. radius in millimeters measured from the center of rotation (center of the centrifuge head) to the bottom of the rotor cavity.
n = speed of rotation (rpm). or use the Nomograph in the graph the rcf of 1000g is 3000 rpm for a radius of 10cm from the centre of rotation

6. Turn the centrifuge off, if not automatic turn off, and allow it to come to a complete stop. Do **not** attempt to open the lid and stop by hand or brake. Remove the tube carefully without disturbing the contents.

***CARE OF THE CENTRIFUGE***

1. If a breakage occurs during centrifugation, switch off the machine.
2. Leave the centrifuge stopped and closed for at least 30 min. to allow any aerosols to settle.
3. Remove the tube holding sockets if any and their contents to a safety cabinet

Repair Personnel:



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Remove the lids (if they are being used) of the tube holder and place the tube holders, lids and tubes into a container for autoclaving or into an appropriate disinfectant, (not into Hypochlorite which will corrode metal).

Disinfect the whole inside of the centrifuge using 70 % Isopropyl / Ethyl alcohol

The operator dealing with the breakage must wear heavy duty gloves with a disposable plastic apron in addition to the conventional protective clothing.

## References

1. Procedures for the Handling and Processing of Blood Specimens, NCCLS, Volume 4, Number 9, 1984, Pages 224–225.
2. General laboratory techniques, procedures and safety. Tietz fundamentals of clinical chemistry . Fifth ed, Saunders Publications, pages 12-14,



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 5. a. MICROBIOLOGY

**Purpose:** Handling of Blood and other samples for Microbiology related tests and procedures

**Scope:** To Perform microbiological tests on various type of samples

**Responsibility:** Lab Incharge/ Microbiology

**Procedure**: as under

| | **Activity/ Description** | **Responsibility** | **Ref. Doc. / Record A1** |
|---|---|---|---|
| | **5.4.1-5.4.9 Refer To Collection Manual** | Technical Staff posted at Collection Counter/Doctor/Medical staff | Master Register |
| | **5.4.10 INSTRUCTIONS FOR REPEAT TESTING** | Technical Staff Counterchecked by Lab Incharge | |
| | Under following conditions repeat tests areperformed:<br>21. When the results are invalid<br>22. When the report of direct staining is not concordant with the culture findings<br>23. On request of clinicians / patient.<br>24. Improper staining<br>25. Abnormal result for cross check | | |
| | **5.4.11 Process Efficiency Criteria OPD and** IPD **(Minimum Desired)**<br>• Repeat Testing <1%<br>• Staining Quality<br>• Culture Media Quality<br>• Compliance to mandatory rules (Bio-Medical | Senior Most Technical Staff Monitored by the Lab Incharge | Should be documented in thequality control register |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | Waste)<br>• Compliance with the Turn Around Time set (as under)<br>• Timely reporting and documentation of Critical Reports as per the annexure | Senior Technician to coordinate with the dispatch staff and monitored by the Lab Incharge | |

| | | | |
|---|---|---|---|
| | **5.4.12 Critical Reports**<br>**Refer to Annexure A2**<br>All the critical reports are informed both telephonically to the concerned doctor and in critical alert movement register maintained by lab. | Technician on duty and Doctors on duty | A Critical Value movement register* is maintained by the lab to the concerned departments (*as per the individual Hospital defined criteria) |
| | **5.4.13 Release of report**<br>All the lab reports are dispatched after final reporting by the lab doctor. The turn around time of the lab reports are<br>• Culture & Sensitivity is 24hrs to 48hrs (Except Blood culture : 2-5days)<br>• Routine Serology 24-48hrs<br>• Special Serology 5-7days. | Technical Staff on duty | Dispatch register is maintained. |
| | **5.4.14 Internal Quality control system**<br>It is a regular process carried out in the lab to standardize the lab system of all sections by-<br>1. By running internal quality control (commercially available) routinely, at the beginning of processing of all the routine samples.<br>2. Repeat runs on the random samples in Serology<br>3. Quality control of Media preparation in-house<br>4. Use of Control ATCC strains | Senior Technician supervised by Department Incharge | Internal quality control registers are maintained. |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **5.4.15 External quality control system**<br>EQAS is maintained by participating in EQAS (External quality assessment system) conducted by Sir Ganga Ram Hospital, Delhi. External quality samples are sent by the institute on quarterly basis and are checked on a marking basis.<br>Reports of assessment are sent to the respective hospital. Annually a Participation certificate is issued. | Senior Technician supervised by Department Incharge | External quality control register and file is maintained. |
| | **5.4.16 Calibrations of equipment**<br>• All the lab equipments are to be calibrated periodically by the company engineer.(annually) | Senior Technician supervised by Department Incharge | A log book and files are to be maintained. |
| | **5.4.17 Validation of reagents, stains, kits etc**<br>• Random re -sampling<br>• By checking the Lot, batch no, and expiry date.<br>• Cross checking of the slides for microscopic findings.<br>• For validation of stains, control slides are checked. | Senior Technician supervised by Department Incharge | Quality Control Register to be maintained. |
| | **5.4.18 System of resolution of complaints and feedbacks from stakeholder**<br>Any lab related complaint and feedbacks are to be resolved by the department incharge and patient satisfaction should be the priority. | Senior Technician supervised by Department Incharge | Complaints and feedback register is maintained |
| | **5.4.19 Referral laboratories (as per the State Government Policy)** | | |
| | 5.4.20 Storage, retaining and retrieval<br><br>Priority should be given to timely disapatch of the reports as per the Turn Around Time.<br>All uncollected/unclaimed Lab reports are to be stored for 1 week only<br><br>**Preferably HIMS system should be in place**<br><br>*Storage of samples*<br>Primary and examination samples<br>• Refer to collection manual | Senior Technician supervised by Department Incharge<br>To be Communicated to the concerned OPD/IPD incharge | Disapatch registers to be maintained Each hospital to set their own disapatch procedure |
| | **5.4.21 Control of documents**<br>• Random checking of the documents<br>• Checking the page numbering<br>• Signing of the documents | Senior Technician supervised by Department Incharge | Register maintained. |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | • Removal of obsolete documents and<br>• Any modification to be done | | |
| | **5.4.22 Procedure for preventive and break down maintenance**<br>• Separate system for collection and handling of sample.<br>• Urgent / instant sampling preferred.<br>• Urgent reporting and dispatching.<br>• Separate register is maintained.<br>• Consultation with treating doctor if required.<br>• Regular information to the Nodal officer dealing epidemic breakdown, through documentation. | Senior Technician supervised by Department Incharge | Separate register for epidemic or breakdown is to be maintained. |
| | **5.4.23 Procedure for internal audit**<br>• All lab stock registers with page numbering are checked time to time.<br>• Lots, batch no of the kits, Date of Expiry and reagents are checked time to time.<br>• FIFO for reagents (first in first out) system is followed. *as per the available stock<br>• All the existing equipments/ machine/ non consumables are checked time to time and tally with the register. | Lab store incharge and Department incharge. | Stock register is maintained. |
| | **5.4.24 Procedures for purchase of external services and supplies**<br><br>As per the hospital procedure and policy. | Purchase Section | |

## Registers

1. Master Lab Register
2. Individual Bench register e.g. Blood Culture, Pus Culture, Serology, Direct Staining etc..
3. Internal Quality Control Register
4. External Quality Register
5. Feedback / Complaint register
6. Critical Value Register
7. Log Books
8. Indent register/Stock Register
9. Bio-Medical Waste Record Register
10. Dispatch Register



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## 1. Alert/ critical values in microbiology

(Prompt reporting and Telephonic messages to Clinicians)

* Positive Blood Cultures/ CSF Gram stain/Cultures

* Positive cultures of MRSA and VRE

* Positive eye cultures growing Pseudomonas aeruginosa

* Positive cultures of pathogenic Neisseriae

* Positive cultures of Group B Streptococcus from a pregnant woman (culture taken at 35-37 weeks gestation)

* Positive Gram stain/ Culture of joints or sterile body fluids

* Positive stool culture for Salmonella/ Shigella/ Vibrio

* Gram stain suggestive of Gas Gangrene

* India Ink Preparation suggestive of Cryptococcus spp.

* ZiehlNeelsen stain positive for Acid Fast Bacilli

* Albert Stain suggestive of Corynebacteriumdiphtheriae

* Hanging Drop Preparation (Stool) suggestive of Vibrio cholerae



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 5.b. 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

**Responsibility:** Lab Incharge//Microbiologist

**Procedure**: as under

| Sr. No. | Activity/ Description | Responsibility | Ref. Doc. Record |
|---|---|---|---|
| | **a) Blood culture and sensitivity**<br><br>PROCEDURE:<br><br>• Take consent from the patient for collection.<br>• Blood sample is collected using a steriledisposable syringe and needle. The vein from which blood is to be drawn must be chosen before the skin is disinfected .Wear gloves.<br>• Cleanse the site with 70% alcohol, then swab concentrically, starting at the middle of the site with appropriate disinfectant {1 to 10% povidone-iodine solution with 0.1 to 1% availableiodine / or chlorhexidinegluconate}.<br>• If the site must be palpated after disinfection, the finger should be disinfected or sterile gloves must be worn<br>• If a person has an existing IV line, the blood should be drawn below the existing line; blood drawn above the line will be diluted with the fluid being infused<br>• Collection of 8-10 ml of blood for culture is strongly recommended for adults. For infants and small children, only 1-5 ml blood can usually be drawn for bacterial culture.<br>• The top of the blood culture bottle is cleanedwith ethanol swab and blood is injected into the bottle aseptically.<br>• The inoculated blood culture bottle is thenshaken in a circular motion to prevent blood from clotting | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **COLLECTION CONTAINER (Annexure)**<br><br>Two types :<br>Adult blood culture bottle<br>Pediatric blood culture bottle<br><br>**Transportation**<br><br>Inoculated blood culture bottles must be transported upright to the laboratory as soon as possible, to avoid chance of contamination.<br><br>**Storage**<br>In case of delay, it should be stored at 37°C in incubator.<br>**Sample Retention Time** : 7days | Doctor/Technical Staff | Master collection register |
| | **b) Urine culture & sensitivity**<br><br>Procedure:<br>• Take consent from the patient for collection.<br>• Patient instructed to wash hand with soap and water, clean the genital area with plain water.<br>• Collect first morning Clean-Catch, Midstream 1-2 ml of urine in a Sterile urine culture container.<br><br>Indoor Patients:<br>• Urine collected aseptically from Indwelling Catheter (The catheter is cleaned with 70% isopropyl alcohol and clamped. With help of sterile syringe and needle, aspirate 1-2ml of urine and collect in sterile container. Remove the clamp.)<br>• Collect suprapubic aspirate in pediatric patients aseptically.<br><br>Transportation<br>Transport within 2 hrs. Keep the sample upright to avoid spillage<br><br>Storage<br>If the sample transportation is delayed beyond 2 hrs then it should be refrigerated (2-8 0C) up to maximum of 24 hrs.<br>Sample Retention Time : 24hrs<br><br>**c) Pus culture & sensitivity**<br><br>Responsibility: Concerned clinical department Doctor | -Lab staff Posted at Collection Counter will hand over the container to patient for appropriate collection.<br><br>-For indoor patients, medical staff of the concerned department. | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Procedure:

- Take consent from the patient for collection.
- Before collecting sample, clean skin surface with 70% alcohol to avoid skin commensal contamination.
- In case of superficial abscess, Sterile swab used for collection. Collect two swabs, one for smear, another for culture. (If possible, avoid cotton swabs, aspiration in syringe is ideal).
- Purulent material aspirated in syringe-in case of deep abscess and transfer in sterile specimen container.

Transportation

- Transport the specimen to laboratory without delay.
- Keep in upright position to avoid spillage.
- Transport media to be used wherever appropriate.

Storage:

Process the specimen within 2 hours. If delay is expected, keep in refrigerator at 2-8 oC.

Rejection criteria:

- Leaking containers/ Open containers.
- Pus sample submitted along with the drainage tube.
- Swabs with little material.
- Dried specimen

Sample Retention Time: 48 hours

**d) Body fluid culture & sensitivity**

(Pleural Fluid, Peritoneal fluid, Ascitic fluid, Synovial fluid)

Responsibility: Concerned clinical department Doctor.

Procedure:

- Take consent from the patient for collection.
- Vigorously cleanse the needle puncture site with 70% isopropyl or ethyl alcohol
- Body fluid specimens are collected by percutaneous aspiration in a sterile disposable container.
- Peritoneal fluid 10ml and other body fluids 5ml is required, but as little as 0.1ml can be processed.
- Culture of large volumes of fluid can be collected in blood culture bottles , will result in a higher



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

yield.

Transportation:

- Specimen should be transported to laboratory without delay .
- Keep in upright position to avoid spillage.
- Transport media to be used wherever appropriate

Storage:

- Specimen should be processed within 1 to 2 hours, if processing delayed should be kept in incubatorat (37 oC).

Rejection Criteria

- Submission of drainage fluids should be discouraged.
- Leaking containers/ Open mouth containers

Sample Retention Time: 48hours

**e) Stool culture & sensitivity**

Responsibility : Patient is instructed by medical staff and container given for collection

Procedure:

- Take consent from the patient for collection.
- Stool (5ml) collected from faeces passed in a clean pan or 1gm / walnut-sized portion of formed stool is collected in a sterile disposable container.
- Fresh specimen needed for recovery of trophozoite.
- Patient instructed not to mix stool sample with urine
- Take care not to soil the rim of the bottle.
- Apply cap tightly.

Transportation

- Specimen should be transported to laboratory without delay.
- Keep in upright position to avoid spillage.

Storage

Specimen should be processed within 2 hours, if processing delayed should be kept in refrigerator at (2-8 oC).

Sample Retention Time: 24hours



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**f) Throat swab culture & sensitivity**

Responsibility: Concerned clinical deptt doctor

Procedure:

- Take consent from the patient for collection.
- While the tongue is kept down with a tongue depressor, a sterile, plain, albumin-coated or charcoal-coated cotton-wool swab should be used to collect exudate from the tonsils, posterior pharyngeal wall and any other area that is inflamed or bears exudate.
- If patient permits, the swab should be rubbed with rotation over one tonsillar area, then the arch of the soft palate, uvula, the other tonsillar area and finally posterior pharyngeal wall.
- Care should be taken not to touch the tongue or buccal surfaces.

Transportation:

- Specimen should be transported to laboratory without delay. Transport media to be used wherever appropriate. Sterile swab in a tube with a cap. (Two swabs, one for gram stain, another one for culture).

Sample Retention Time: 24hours

**g) Sputum culture & sensitivity**

Responsibility: Doctor/Sister will instruct the patient for collection of coughed out sputum.

Procedure:

- Take consent from the patient for collection.
- Collect the sputum when the patient first coughs on waking in the morning.
- Expectorated Sputum- deeply coughed specimen, expelled into a wide-mouthed sterile container, with an attempt to minimize contamination by saliva.
- Induced sputum – rinse patient's mouth with sterile water or saline. Using an ultrasonic nebulizer, have the patient inhale approximately



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | 20 to 30 ml of 3% NaCl . Collect induced sputum in a leak proof container.<br>Transportation<br>• Specimen should be transported to laboratory without delay<br>• Keep in upright position to avoid spillage.<br>Storage<br>• Specimen should be sent to the laboratory without delay. If delay is anticipated, it should be kept in refrigerator at 2-8 oC.<br><br>Rejection Criteria<br>• Saliva unacceptable<br>• Leaking containers/ Open mouth containers<br>• Specimen is dried up.<br><br>Sample Retention Time: 24 hours<br><br>**h) Cerebrospinal fluid culture (csf) and susceptibility testing**<br><br>Responsibility : Concerned clinical department Doctor.<br><br>Procedure:<br>• Take consent from the patient for collection.<br>• CSF samples collected by lumbar puncture under aseptic conditions.<br>• Lumbar puncture site is cleaned with 70% isopropyl alcohol followed by tincture of iodine to kill surface and subsurface bacteria<br>• Remove the tincture of iodine with 70% isopropyl alcohol. It is left for 1-2minutes to dry.<br>• About 3-5ml of CSF is collected in a fresh sterile screw capped container.<br><br>Transportation:<br>• The specimen must be dispatched to thelaboratory as quickly as possible, for delay may affect the recovery of delicate pathogens and result in disintegration of leucocytes.<br>• The specimen must be transported upright to the laboratory as soon as possible, to avoid chance of contamination<br><br>Storage :<br>If delay of few hours is unavoidable, the specimen is best | | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | kept in an incubator at 37°C.<br>Do not keep the sample in refrigerator.<br><br>Sample Retention Time: 48hours<br><br><br>**i) High vaginal swab culture & sensitivity**<br><br>Responsibility: Concerned clinical department doctor.<br><br>Procedure:<br>• Take consent from the patient for collection.<br>• A sterile swab is inserted into the upper part of the vagina and rotated there before withdrawing it , so that exudate is collected from upper as well as lower vaginal wall.<br>• Two swabs should be collected one for gram stain and another for culture.<br><br>Transportation:<br>Specimen should be transported to laboratory without delay.<br><br>Storage:<br>Specimen should be processed within 2 hours, if processing delayed should be kept in refrigerator at (2-8 0C).<br><br>Sample Retention Time: 24hrs. | | |

References

**1.** Basic laboratory procedures in Clinical Bacteriology, 2nd Edition, World Health Organization Geneva, 2003.

**2.** Topley & Wilson's Microbiology & Microbial Infections 9th Edition

**3.** Mackie and McCartney Practical Medical Microbiology, 14th ed. Collee JG, Fraser AG, Marmion BP, Simmons A, Eds. (Churchill Livingstone) 2008

**4.** Forbes BA, Sahm DF, Weissfeild AS. Bailey and Scott's Diagnostic Microbiology: 12th ed. (Elsevier) 2007

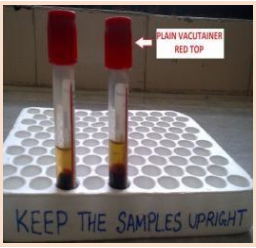
**5.** Clinical Microbiology Procedures Handbook, Henry D. Isenberg, Lynne S. Garcia, volume 1, 2nd Edition Update (2007).

Annexure

1) **Containers for collection of various microbiological samples**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| S.No | Type of container | | Type of sample/test |
|---|---|---|---|
| 1 | Plain Vacutainer (Red Cap) |  | Serological Tests<br>•Widal<br>•ASO<br>•CRP<br>•HBsAg<br>•HCV<br>•Dengue ELISA<br>•Others |
| 2 | Sterile Plastic Containers |  | Culture & Sensitivity<br>•CSF C/S<br>•Pus C/S<br>•Urine C/S<br>•Pleural Fluid C/S<br>•Sputum C/S<br>•Others |
| 3 | Blood Culture Bottle |  | Blood Culture & Sensitivity<br><br>Adult Samples (Big)<br>Pediatric Samples (Small) |
| 4 | Sterile Disposable Swab |  | Pus Swab, High Vaginal Swab C/S |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 6. CYTOLOGY

**Purpose:** Collection and Handling of samples in cytology section

**Scope:** To define the cytology procedures and its handling

**Responsibility:** Lab Incharge/ Pathologist

**Procedure**: as under

| Sr. No. | Activity/ Description | Responsibility | Ref. Doc. / Record |
|---|---|---|---|
| | **Collection and handling of primary sample**<br>In general, material for cytological examination is obtained by:<br>1. The patient is sent to the Fine Needle Aspiration Cytology (FNAC) clinic for aspiration of visible, surface lumps.<br>2. The smears received in the Lab either in the form of smears prepared by examining cytologist, physician, gynecologist, surgeon or their assistants at the time of clinical examination e.g. cervical smears, fnac smears, guided fnac smears, smears for review from outside lab<br>3. In the form of fluid specimens which are forwarded to the laboratory for further processing e.g. Body fluids such as<br>➢ Pleural fluid<br>➢ Ascitic fluid<br>➢ Peritoneal fluid<br>➢ Pericardial fluid<br>➢ Joint fluid<br>➢ Cystic fluid (Breast/tumor)<br>➢ Sputum<br>➢ Urine<br>➢ Cerebrospinal fluid (CSF)<br>➢ Gastro-intestinal aspirates etc.<br>➢ Endometrial aspirates | Technical Staff posted at cytology lab<br><br>To be checked by I/C from time to time | CYTOLOGY Master register |
| | ➢ The appointments may be given on scheduled date and time with proper instructions<br>➢ I/C needs to monitor that unnecessary delays don't happen and priority to urgent cases are given ( criteria for urgency needs to be defined in every hospital) | Technical Staff posted at cytology lab<br>To be checked by I/C from time to time | Appointment register |
| | **6.4.2 Procedure for transportation of primary sample with** | Nursing staff | Register at |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **specification about time frame, temperature and carrier**<br>1. Spray fixatives whenever the sample is to be transported- contain 2-10% carbowax in 95% alcohol.<br>2. Protect the smears from drying by forming an invisible film on the surface of the slides<br>3. May be used in lieu of fluid fixatives i.e. immediately after the process of smear preparation has been completed.<br>4. Correct use of spray fixative calls for several precautions such as:<br>i. Spray must be smooth and steady<br>ii. Distance between spray nozzle and smear must be 10-12 inches (25-30 cm)<br>Smears coated with spray fixatives must be air-dried before mailing | Posted, supervised by doctor i/c | Point of origin i.e. OPD/IPD |
| | **6.4.3 Points of acceptance and rejection of primary samples**<br>ii) Points of acceptance of sample where Documentation of<br>• site of receipt of sample<br>• date and time of sample collection<br>• date and time of sample receipt.<br>• amount of fluid received.<br>• gross appearance of the fluid.<br>• total number of smears prepared.<br>• total number of dry smears.<br>• total number of clot smears (if any).<br>• transfer the details to the cytopathology register.<br>• transfer the details on a form, which will be forwarded with the slides for reporting. | Technical Staff posted at cytology lab | Cytology Master register |
| | **6.4.3( a**) Process on rejection of primary samples<br>• Incomplete form w.r.t demographic data, clinical details<br>• Inadequate material<br>• Hemorrhagic material<br>• Improper fixation | Technical Staff posted at cytology lab | Master register |
| | Procedure of FNAC( see annexure)<br>Fixation (see annexure ) | Senior resident assisted by Staff posted and supervised by Section I/C | **See Annexure 1 &3** |
| | Fluid specimens(see annexure ) | Technical Staff posted | **See Annexure** 8, |
| | Staining Procedure(see annexure ) | Technical Staff | **See Annexure** 7-11 |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **6.4.4 Release of reports**<br>Fluids on same day<br>FNAC within 2-3 days | Technical Staff posted<br>Supervised by senior resident | |
| | **6.4.5 Repeat test due to analytical failure**<br>Scant or inadequate aspirates<br>For special stains and immunohistochemistry | senior resident supervised by doctor i/c | |
| | **6.4.6 Storage of examined samples and maintenance of records**<br>Smears of FNAC stored for 5 years<br>Fluids can be stored in refrigerator for 24 hours<br>Records are maintained as per the guidelines<br>Index register maintained for quick access of record. | Technical Staff posted<br>Supervised by senior resident and supervised by Section I/C | Discarding register |
| | **6.4.7 Quality control**<br>**Internal quality control**- regular checking of routine staining, control lot of smear stained with special stains | Technical Staff posted<br>Supervised by senior resident and supervised by Section I/C | Quality control register |
| | **6.4.8 External quality control-**<br>WITH TATA MEMORIAL Hospital. Mumbai/ IAC or as per availability | Section I/C | EQUAS register |
| | **6.4.9 Calibration of Equipments:**<br>**1. Centrifuges**<br>**2. Cytospin** | Senior Technician | |
| | **Validation of reagents, stains etc**<br>Lot verification<br>Positive control | Senior Cytology Technician) posted<br>Supervised by senior resident and supervised by Section I/C | Stock register |
| | **6.4.10 Resolution of Complaints**<br>Any lab related complaint and feedbacks are to be resolved by the department incharge and patient satisfaction should be the priority. | Technical Staff posted<br>Supervised by senior resident and supervised by<br>Section I/C / grievance officer of the department | Complaints register |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | **6.4.11Referral Laboratories –**<br>(As per the State Government Policy).<br>Representative Slides may be issued to the patient when referred to Higher Center for further management. (On Demand) | Technical Staff posted<br>After request is verified by Section I/C | |
| | **6.4.12 Storage, retaining and retrieval of records**<br>• Priority should be given to timely dispatch of the reports as per the Turn around Time.<br>• All uncollected/unclaimed Lab reports are to be stored for 1 month<br>• **All stained slides are to be preserved for 5 years**.<br>**Preferably HIMS system should be in place** | Technical Staff posted<br>Supervised by senior resident and supervised by Section I/C | Cytology Master register<br>Discarding /weeding out register |
| | **6.4.13 Control of documents-**<br>• Random checking of the documents<br>• Checking the page numbering<br>• Signing of the documents<br>• Removal of obsolete documents and any modification to be done<br>• Random Checking for report entries in the master register<br>• Indexing | Senior Cytology Technician supervised by Senior Resident | Work supervision register maintained by doctor I/C<br>Indexing register |
| | **6.4.14 Internal Audit**<br>**(with performance Monitoring Criteria)**<br>**DETAILS IN ANNEXURE**<br>• Pap Smear related – Set the upper limit for ASCUS /CINI<br>• Staining Quality<br>• Inter Observer Variability<br>• Incidence of Repeat aspiration in case of inadequate samples<br>• Histopathological correlation | Doctor I/C | Quality control register Annexure 12 |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## References

3. Orell's And Sterrett's – Fine Needle Aspiration Cytology 5th Edition
4. Koss Diognostic Cytology And It's Histopathologic Bases 2006 Edition

**Registers**

1. Appointment register
2. Cytology master register
3. Quality control register
4. Work supervision register
5. Discarding /weeding out register
6. Stock register
7. EQAS Register
8. Indexing Register As Per Hospital Policy (**Preferably HIMS** Needs To Be In Place)
9. Complaints register

## Annexures

### 1. Procedure of FNAC

1. Patient Is Called On A Designated Day
2. Demographic details are checked for age, sex, CR No., date, clinical details and clinical provisional diagnosis
3. The procedure is explained to the patient and consent is taken for the same
4. Make the patient aware of the simplicity and noninvasive nature of the FNA technique make him/her relaxed & non-apprehensive.
5. Instruct the patient to lie down on the couch and patient is made comfortable.
6. Material required for FNAC - Syringe with needle (10 cc disposable syringe, disposable needles of different gauge : 22 G,23G, 24G), glass slides, diamond pencil, fixatives (100% methanol, 95% ethanol)
7. Lesion is fixed with left hand
8. Site examined and cleaned with standard protocol
9. Lesion is examined for size, feel, depth, tenderness and provisional impression
10. Negative pressure is exerted and aspirated till material in hub.
11. Material is pushed on clean numbered slide smears prepared as per protocols and fixed as per protocol and stains decided
12. All pus samples for GIEMSA/H&E/ PAS/ AFB/ unstained smears
13. All suspected cancers for H/E; PAP;/Giemsa/;
14. Cold acetone fixed slides for immunostaining as per availability*.
15. 2 unstained fixed smears for some stains to be decided later.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

## 2. Preparation of smears

1. For most diagnostic purposes, well-prepared and well-fixed smears are required.
2. Air-drying of smears should be avoided, if prepared for wet fixation.
3. Monolayer preparation is suitable for almost all processing techniques.
4. Considerable skill and practice are required to prepare excellent smears by single swift motion without loss of material or air-drying.
5. Excessive crushing of the material must be avoided.
6. A competent help must be secured in advance.

## 3. Fixation

1. Immediate fixation of smears is essential for the correct interpretation.
2. Air dried smears are required or desirable in special situations (special stains e.g. MGG stain) for wet fixation.
3. Most of the fixatives are alcohol-based e.g. 95% ethanol, 95% rectified spirit, 80% iso-propanol or propanol, absolute methanol, Ether+95% Ethanol mixture (1:1), Carnoy's fixative

For Cervical and other FNA smears or in koplin jar.

For spray fixative

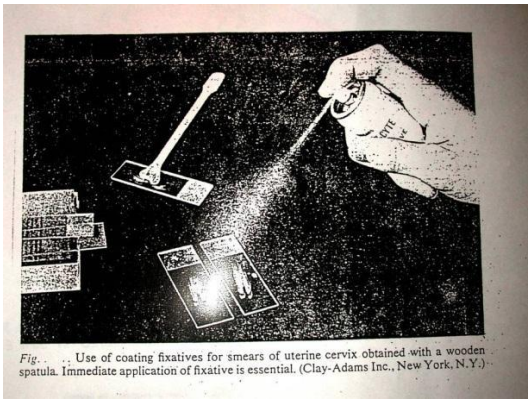


*Fig. . .* Use of coating fixatives for smears of uterine cervix obtained with a wooden spatula. Immediate application of fixative is essential. (Clay-Adams Inc., New York, N.Y.)



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

4. Fluid specimens

1. May be obtained from a variety of body sites such as:
   i. Respiratory tract (sputum)
   ii. Gastro-intestinal tract (endoscopic aspirates)
   iii. Urinary tract (Urine samples)
   iv. Effusion fluids (body cavity fluids-pleural/ peritoneal/pericardial) should be collected in
2. Anticoagulants container
   i.(1% ammonium oxalate in the ratio of 9:1 i.e. 9 parts of fluid and 1 part anticoagulant)
   ii. Heparin

## 5. Procedure for processing body fluids

Aim: - To obtain pleural, peritoneal, pericardial and cerebrospinal fluids for fluid cytology. Usually look for malignant cells and cell type.

Preferred sample

- Fluids received in leak proof sterile containers.
- Fresh samples recommended.
- In case of transport delay anti coagulant – 3.8% Sodium Citrate or EDTA to be used to prevent clot formation.

Materials

- Leak proof containers
- Fixative
- Ordinary Centrifuge
- Cytospin filter paper strips*(as per availability)
- Cytospin Centrifuge*
- Glass tubes
- Glass slides
- Staining solutions

Procedure

- Receive the sample with positive identification of the patient.
- Centrifuge the fluid at 1500 RPM for 5 minutes.
- Two sets of smears to be prepared from the sediment.
  - Air dried smears for Rapid H&E stain and AFB stain (if necessary).
  - Wet fixed smears for PAP stain



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Procedure for Scanty & clear fluids

- Label the slide
- Cover the slide with the filter paper
- Fix the slide in the cytospin bucket.
- Pour the sample till the marking on the bucket.
- Centrifuge at 500 RPM for 5 minutes.

- Slides are removed from the bucket with the cell button on it.
- Two sets of smears to be prepared from the sediment.
    - Air dried smears for giemsa stain and AFB stain(If necessary)
    - Wet fixed smears for H&E/ PAP stain
    - microscopic examination

## 6. Microscopic Examination of Body Fluids

**Requirements**:

- Glass slides
- Neubauer's counting chamber
- Pasteur pipette
- Diluting fluid( turk's fluid)
- Gram's staining reagents
- Acid –fast staining reagents
- Giemsa staining reagents
- Centrifuge machine
- Microscope.

**Procedure:**

1. Mix equal volume of body fluid and diluting fluid carefully.
2. Fill the one side of the neubauer's chamber with the above mixed fluid and other side directly with the body fluid.
3. Leave the chamber for 5 minutes to allow the cell to settle.
4. Place the chamber on the microscope.
5. Cells counted in all the 9 squares.
6. Calculations

   Direct:-$\frac{\text{cells counted x 10}}{9}$



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Indirect ie. After dilution 1:20

$$= \frac{\text{cell counted } x10 \ x\ 20}{9}$$

7. If required make a thin smear of the sediment.

8. dryand fix it .

9. Stain by giemsa stain / gram's stain / acid fast bacilli stain.

Quality Control: Compared with internal quality control slides

**Staining procedure-as under**

1. Two sets of smears to be prepared from the aspirate.
    - Air dried smears for giemsa stain and AFB stain when clinically indicated.
    - Wet fixed smears for H&E, PAP stain.
2. Quality Control: Compared with internal quality control slides
3. First slide of the everyday's batch to be checked for quality of staining

## 7. Procedure for H&E staining

Purpose: Routine stain for FNA smears.

Principle: Hematoxylin is a dark blue or violet stain that is basic and binds to DNA, RNA. Eosin is a red acidic stain and bind to positively charged proteins, intermediate filaments etc

**Procedure**

- Wet Fix the smear in 95% ethanol
- Dip the slides in hematoxylinfor 15 minutes.
- Rinse in water.
- Dip the slides in 1% Hcl for 1-2 min
- Wash in running water for 5 min.
- Dip the slides in eosin for 1 min.
- Wash the slides in acetone.
- Clear the slides in xylene
- Mount with DPX.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

Quality Control: Compared with internal quality control slides

## 8. Procedure for giemsa staining

**Purpose**: Routine stain of FNA smears.

**Principle**: Giemsa solution is a mixture of methylineblue, eosin, and Azure B. The stain is usually prepared from commercially available Giemsa powder.

**Procedure:**

- Air dry smear is fixed in pure methanol for 30 secondsor ten dips
- The smear slides are covered with 20-25 % Giemsa stain for 10- 12 min.
- Then fiushed with tap water and left to dry
- Quality Control: Compared with internal quality control slides

## 9. Procedure for Z. N. Staining

Purpose: To stain TB bacilli in the sputum samples and FNA smears.

Principle: By this method bacteria may be divided into 2 categories depending on their ability or inability to resist decolourisation by acid and alcohol. The TB bacilli resist decolourising by acid and alcohol. It will remain pink while other organism and material take blue colour

**Procedure**

- Fix the smear by air dry
- Flood the smear with strong carbolfuschin for 10 minutes.
- Decolorize with 3% acid alcohol for 1 min.
- Rinse in tap water.
- Pour Methylene blue on the smear and keep it for 3 minutes.
- Wash in tap water.
- Dry the smear
- Mount with DPX.

Quality Control: Compared with internal quality control slides

## 10. Procedure for mucicarmine staining (as per availability)



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**Purpose**: To stain MUCIN in smears, and encapsulated fungi

**Principle:** Aluminium is believed to form a chelation complex with the carmine, changing the molecule to a positive charge allowing it to bind with acid substrate of low densities.

**Procedure**

- Wet Fix the smear in ethanol
- Hematoxylin for 10 minutes.
- Rinse in running tap water for 5 mins.
- Mucicarmine solution for 30 seconds
- Wash in tap or distilled water.
- Metanil yellow, 30 seconds to 1 min
- Dehydrate in three changes of ethanol and clear in xylene.
- Mount with DPX.

Quality Control: Compared with internal quality control slides

## 11. Procedure for papanicolaou staining

**Purpose**: To differentiate cells in smears preparations of bodily secretions
Pap stain involves five dyes in three solutions
Hematoxylin- nuclear stain
Orange G 6- for keratin
EA (eosin azure)
EY- for mature squamous cell, nucleoli, cilia, RBC
Light green SF yellow stain for cytoplasm of metabolically active cells
Bismark brown

**Procedure**

- Wet Fix the smear in 95% ethanol(6 dips)
- 70% alcohol(6 dips)
- 50% alcohol (6 dips)
- Rinse in water
- Hematoxylin for 10- 15 minutes.
- 1% Hcl for 1 minute
- Wash with tap water
- 50% alcohol (6 dips)

- 70% alcohol (6 dips)
- 9 5% alcohol (6 dips)
- PAP solution for 20- 30 minutes
- 95% alcohol (6 dips)
- 95% alcohol (6 dips)
- EA50 for 20-30 minutes
- 95% alcohol (6 dips)
- 95% alcohol (6 dips)
- 70% alcohol (6 dips)
- Clear in xylene.
- Mount with DPX

Quality Control: Compared with internal quality control slides

## 12. Gram Staining

**Purpose** : To stain bacteria

**Reagents**:

- Crystal violet stain
- Gram's iodine solution
- Acetone
- Basic fuchsin stain
- 0.1 % picric acid in acetone

**Specimen**: Pus , Body Fluid

**Equipment**: Glass slides,Spirit lamp, Pipettes

**Procedure**

1. Flood the slide with the freshly prepared solution of crystal violet stain for 1 minute.
2. Rinse in water.
3. Flood with Gram's iodine solution for one minute. Rinse with distilled water and blot to dryness by using filter paper.



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

4. Decolorize with acetone briefly (Until no more blue colour runs off)
5. Rinse immediately in tap water.
6. Stain for 1 minute with 0.1%(w/v) basic fuchsin.
7. Wash in water and blot gently.
8. Dip and rinse briefly in acetone and xylene equal parts.
9. Clear and mount.

**Results**

| | |
|---|---|
| **Gram positive bacteria** | **Blue** |
| **Gram negative bacteria** | **Red** |
| **Nuclei** | **Red** |

## 13. Aims / scope of Internal Quality Control for Cytology

Accuracy of screening must be monitored and managed with approved protocols and procedures for defining and dealing with poor performance. Internal quality control of cytology screening must be monitored by:

• Re-screening of slides initially judged during primary screening as negative or inadequate to detect false positives/negatives and to determine sensitivity and specificity rates

• Monitoring screening detection and reporting rates by measuring the percentages of the main types of cytological findings (high grade, low grade, inadequate, undetermined, negative) detected by individual screeners and cytopathologists, and in comparison with the laboratory as a whole, the programme and national standards

• Performance evaluations to identify those with deficiencies in knowledge and skills who would benefit from a more directed educational programme

• Correlation of cytology with clinical/histological outcomes

• Re-screening of samples from women with negative or low grade test results less than 3 or 5 years before diagnosis of invasive cancer

• Correlation of cytology with HPV testing for smear tests reported as ASCUS



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

# 7. HISTOPATHOLOGY

(Where specimens are being received and sent to referral laboratories)

**Purpose:** Collection and Handling of samples in histopathology section

**Scope:** To define the basic handling of specimens

**Responsibility:** Lab Incharge/ Pathologist

**Procedure**: as under

| Sr. No. | Activity/ Description | Responsibility | Ref.Doc. / Record |
|---|---|---|---|
| | **Collection and handling of primary specimens** The biopsies/ specimens are generated in the Minor OT/Major OTs<br>Also are received Blocks and slides for review.<br><br>The pre requisites are-<br>a. Filling up of requisition forms<br>b. Container with 10% FORMALIN to be kept ready by OT/Nursing staff<br>c. To ensure that fixatives is 10 times the volume of primary sample.<br>d. All these details should be uploaded on HIMS where ever available. | Nursing sister OTs | |
| | 7.4.1.1<br>A) Specimens are transported to the laboratory by the designated staff with entry of the –<br><br>1. details of the patient viz name, age, sex ,patient id no,<br>2. sample details viz site, side, specimen description,<br>3. clinical details, relevant history ,<br>4. Collection date<br>5. submitting physicians name and stamp etc. on the requisition form<br><br>The forms to be submitted in duplicate.<br><br>A) CONTAINER AND ITS LABELLING- | Nursing sister OTs, supervised by senior resident – concerned speciality surgical department | |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | The container should have an opening larger enough so that the tissue can be removed easily after it has hardened by fixation.<br><br>The container should be properly sealed to prevent any leaks.<br><br>The container labels must be legibly labelled with the following-<br>4. At least two patient identifiers- the first identifier must be the patients name and second must be the medical record number/ age and sex.<br>5. The anatomical source of each specimen on each container. | | |
| | **7.4.2 Transportation of samples-**<br>Specimen should be transported in glass, plastic or metal container or in a plastic bag in 10% formalin (10 x volume of specimen size) and these should be labelled properly.<br>The sample should be transported preferably on the same day.<br>The samples should be transported at room temperature.<br>Sample brought by the staff is logged into their handling register, signed by the sister in charge. | Nursing sister OTs, supervised by senior resident–concerned speciality surgical department | Dispatch register of respective OTs<br><br>Specimen receiving /handling register |
| | **7.4.3 Acceptance and rejection of the samples-**(if the samples are being sent through pathology labs )<br>Receipt of such specimens does not necessarily precludes its histologic examination, but may limit its accessibility | Technical Staff posted Counterchecked by senior resident<br>The resident must document the specimen condition as it was received and what corrective action was taken. | Master register, incident reporting register histopathology<br><br>**Annexure - 1** |
| | **7.4.4 Labelling of the samples**<br>PATHOLOGY CASE NUMBER-<br>At accessioning, a Pathology number is assigned to the patient sample. This becomes the unique identifier for the patient, sample and date of procedure.<br>This number is placed on all requisition slips, sample container, tissue cassettes, blocks and slides. | Technical Staff posted | |
| | **7.4.5 Repeat tests due to analytical failure –** | The grossing resident | log/adverse |



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

| | | | |
|---|---|---|---|
| | The log of such cases is maintained in log /adverse event register.<br><br>Possible causes-<br>a. Inadequate fixation<br>b. Burnt tissue<br>c. Non- representative sampling<br>d. Inadequate biopsy<br>e. Mismatch demographic details.<br><br>. | and hisopathology Staff will work together to identify such cases and their rectification.<br>The resident must quick page/ contact the appropriate staff to get the missing forms/ details regarding the same<br>The residents or attending pathologist must be consulted if there is a question about matching source description. | event register . |
| | **7.4.6 Procedure of report disapatch (if the samples are being sent through pathology labs )**<br>The referring and referral labs should maintain a close collaboration regarding TAT, receiving anddisapatch of samples and reports.<br>The final report as received from the referral lab will be issued to the patient after checking the patient's details from the referring lab | | |
| | **Process Efficiency Criteria**<br>• Less than 1% autolysed<br>• Patient Complaints<br>• Wrong Labelling<br>• Delays<br>*Each hospital may define their own criteria. | Sister Incharge<br><br>Doctor On Duty<br><br>Resident doctor incharge<br><br>Senior Most Technical Staff (1-3) will communicate to the sister in charge Ward/IPD<br><br>Monitored by the Lab Incharge | |

**This SOP is only for centres where specimens are being received and sent to referrallaboratories. Other centres where grossing, processing and reporting is being done will make necessary changes.**



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

**References – Bancroft's Theory and Practice of Histological Techniques**

## Annexures

### 1. Acceptance and rejection of the samples-

ACCEPTANCE-

Technical staff in the laboratory checks the labels on the container and match them with the requisition form.

It is also checked if the container is leak proof

Is there adequate formalin

REJECTION OF SAMPLES:

- All cases must be accompanied by requisition forms duly filled, verbal requisition is not accepted.
- Incompletely filled form
- Improperly labelled containers
- Details on form and sample container not matching
- Mismatching demographics.
- Tissue not sent in formalin or inadequate amount.
- No tissue in the container

SPECIMEN QUALITY PROBLEM-

Such problems include-

1. Inappropriate fixation
2. Delayed fixation
3. Delayed delivery
4. Leaking container
5. Dessication
6. No tissue seen
7. Badly damaged blocks
8. Broken slides
9. Understainedslided

INCIDENT REPORTING



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow

- Any problem as stated above must be brought to the notice of the Histology supervisor.
- The supervisor is responsible for reporting such problems in the quality improvement and assessment log.

## 2. How to make 10% formalin-

Making 10% Neutral Buffered Formalin from stock solutions Where only a standard stock solution of formalin* is available it is typically 37-40% formaldehyde (a gas) in aqueous solution and unbuffered.

To make a histological fixative from this we need a 10% solution** of this stock formalin i.e. 1 part of the stock formalin with 9 parts water, preferably distilled. This makes an unbufferedformalin solution, which will have a pH of 3-4. If used unbuffered the acidity can react with haemoglobin in the tissues to produce dark brown acid formaldehyde haematin precipitates, which complicate histological interpretation.

To adjust the 10% formalin solution to a neutral pH mix in quantities of a buffer, typically sodium phosphate.

A recommended recipe is as follows: 100ml Formalin (37-40% stock solution) 900ml Water 4g/L $NaH_2PO_4$ (monobasic) 6.5g/L $Na_2HPO_4$ (dibasic/anhydrous) 10% formalin can also be referred to as formal or formol.

* Formalin is formaldehyde gas dissolved in water and reaches saturation at 37-40% formaldehyde. This can therefore regarded as 100% formalin

** 10% formalin actually represents10% of the 37-40% stock solution. The actual amount of dissolved formaldehyde in the 10% formalin is therefore only 3.7-4.0%



Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow


**Sanjay Gandhi Post Graduate Institute of Medical Sciences**
**Lucknow**

Lt Col Varun Bajpai VSM
Executive Registrar
SGPGIMS,Lucknow