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Central Laboratory Manual

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Scope of the Document

This manual provides information on Central Referral Hospital-Central Laboratory (CRHCL), scope of services, sample collection procedures, information on all the tests done in CRHCL w.r.t. sample collection & transport, clinical utility, turnaround time and safety aspects. The manual is for the use of all laboratory staff, patients, clinicians and nursing staff. Hard copies of the manual are made available in OP sample collection Centre for the users to refer. Soft copy of the manual is made available to in house use only, in the Backbone HIS. This manual is prepared based on the guidelines of ISO 15189:2012, NABL 112, WHO.

1. INTRODUCTION

The Central Referral Hospital (CRH), is a 550 bed Tertiary care Hospital that caters to about 1200 outpatients and 60 new admissions as In-Patients daily. CRHCL caters about 200 samples every day. The lab offers its diagnostic services to Patients attending Out Patient Department as well as to the In-Patients. CRHCL comprises of following sections: Clinical Biochemistry, Haematology, Clinical Pathology, and Microbiology (Bacteriology, virology, serology and mycology). The lab has a sample collection centre for outpatients.

Vision of CRH:

To be a world class centre for health care.

Mission of CRH:

to cure with care, courtesy, compassion, and competence.

Vision of CRHCL:

To be a superior provider of medical laboratory services at reasonable cost but highest quality

Mission of CRHCL:

To provide high quality medical laboratory services with continual quality improvement.

2. GENERAL INFORMATION

2.1 Contact Details:

2.1.1 Postal address: central referral hospital, 5th mile Tadong 737102, east Sikkim, Sikkim

2.1.2 Website of smims: https://smu.edu.in/smims/hospital.html

2.1.3 Telephone: Administrator, CRH: 03592 232 041, ext. 116, 117, 124

2.1.4 Telephone number for labs:

OP sample collection/ IP sample reception/op sample billing counter: 03592 232 041, ext. 165

Clinical biochemistry: 03592 232 041, ext. 226

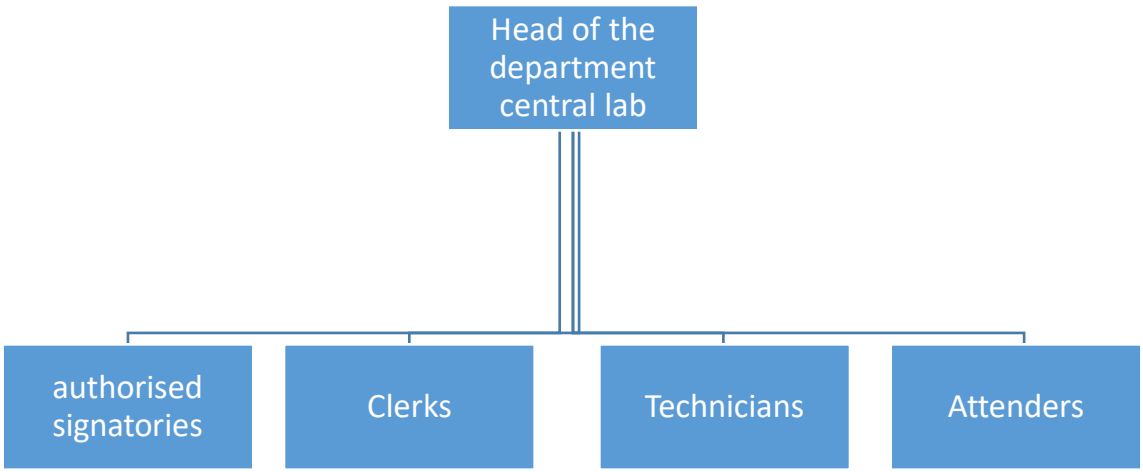
Haematology and clinical pathology: 03592 232 041, ext. 164

Microbiology: 03592 232 041, ext. 155

2.2 Lab timing:

Department / Activity	Working Days	Opening hours
Lab reception/sample collection centre	**Monday to Saturday**	**8 a.m. to 5 p.m.**
biochemistry	**Monday to Sunday**	**24 hours**
pathology	**Monday to Sunday**	**24 hours**
microbiology	**Monday to Sunday**	**24 hours**

2.3. Organogram of Lab:



2.4. Lab Charges: Charges for tests done in CRHL are mentioned in schedule of charges available in hospital enquiry, billing and OP sample collection centre. Electronically, charges are available in billing section.

2.5. User feedback :

2.6. Availability of User Information Materials:

1) Directory of services and Sample Collection Manual- is available as a hard copy in OP sample collection center and Lab Reception, for the use of lab staff, clinicians and nursing staff, and also for the other users to refer. Electronic copy is available for the use of lab staff, clinicians and nursing staff in "Backbone HIS".

2) Standard operating procedure (SOP) of sample collection - are available in OP sample collection centre.

3) Request Forms: List of tests done and brief information on samples is available. The laboratory has a number of different request forms. They are used for different Laboratory analyses. It is important that the correct form is supplied for a particular test. Following are the types of request forms available: Form for Biochemistry; Haematology; Microbiology; and Multipurpose form.

4) Displays in the OP sample collection centre - provide quick information to users on general instructions to be followed.

2.7. Advisory Services of CRHCL:

CRHCL consultants (Biochemists, Pathologists and Microbiologists) are available for consultation if the patients ask for any advisory services.

2.8. Ethics in CRHCL:

- CRHCL follows ethical principles of autonomy, justice, beneficence and non-maleficence. CRHCL and its individual sections offer its services keeping the patients welfare as its paramount interest. The patient's right to privacy and confidentiality of data /lab reports are also important to the practice of laboratory medicine. The information gathered from the patient and the records created about the patient are for his / her benefit. The information will not be disclosed to a third party without the patients consent unless it is a medico legal case.
- Staff collect the basic information from patients, which include name, sex, age, hospital ID, status of fasting/postprandial, medications, which are required for correct identification of patients and interpretation of results. No unnecessary personal information is collected. The reason for collection of information is explained to the patient.
- Adequate information is provided to the patient in an understandable language, on sample collection procedure and the tests to be performed. Written informed consent is taken from the patients/care givers for HIV testing.
- Adequate privacy is provided in the sample collection area, appropriate to the type of specimen collected.
- The patients are given information about the turnaround time of tests requested. In case of any delay due to unexpected circumstances, the patients are given information.
- Patients are treated with respect and courtesy by the staff of CRHCL.
- CRHCL entertains any complaints from users.
- The consultants in CRHCL are available to provide any advisory service to patients on tests and interpretation of results.
- Any tests which cannot be carried out by the lab under appropriate standard conditions are refused by the laboratory and informed to the patient before collection of samples.
- In case of tests which are not performed by CRHCL and in unforeseen circumstances such as instrument breakdowns, wherever possible CRHCL takes responsibility of sending the samples to a referral laboratory. CRHCL takes responsibility of informing the patients for such procedures.
- CRHCL takes care of patient safety.
- The biomedical waste in sample collection areas (needles, syringes, gloves, infected paper towels/tissue papers, cotton, etc.) is disposed in accordance with standard guidelines. CRHCL has ensured good housekeeping practices in sample collection area, waiting area and toilets.

2.9. Tests Available in CRHCL:

The tests done in CRHCL are available under following lab sections:

1) **Clinical Biochemistry** : All routine tests including glucose, lipid profile, renal function tests, liver function tests, enzymes, cardiac markers, electrolytes, arterial blood gas analysis, and various special tests – hormones, vitamins & tumour markers, specific antibodies.

2) **Hematology & clinical pathology**: CBC with peripheral blood smear report, Absolute eosinophil count, Reticulocyte count. ESR. Urine routine and microscopy,

3) **Microbiology**: Serology of viral markers, Stool examination, RF,CRP,ASO, Tuberculin test, Malarial antigen test, WIDAL,VDRL.

2.10. Referral Labs : The tests which are not done in CRHCL are outsourced to referral labs. CRHCL has a documented procedure for selecting and evaluating referral laboratories. CRHCL selects the referral labs based on their criteria such as accreditation of referral lab by accrediting agencies (NABL, CAP), implementation of quality control programme in the referral lab, logistics to the referral lab and reputation of the lab. CRHCL is responsible for selecting a referral laboratory. Suggestions from clinicians are taken into consideration in selecting referral laboratories. CRHCL is responsible for sending samples to referral labs and for providing test results. This process is not hindered by financial or commercial considerations. Any tests which cannot be carried out by the lab under appropriate standard conditions are refused by the laboratory and informed to the patient before collection of samples. In case of tests which are not performed by CRHCL and in unforeseen circumstances such as instrument breakdowns, wherever possible CRHCL takes responsibility of sending the samples to a referral laboratory. CRHCL takes responsibility of informing the patients for such procedures.

2.11. Turnaround Time:

For outpatient tests, the turnaround time (TAT) is the Time from Sample collection to Result availability after signature from authorized signatories. For Inpatient tests, TAT is from receiving the sample in the lab to availability of provisional results. For urgent test requests, TAT is shorter (STAT). The TAT and STAT of tests are mentioned later for all tests in CRHCL.

2.12. Quality Control in CRHCL:

CRHCL has an internal quality control programme for most of the tests done. CRHCL participates in external quality control programmes to ensure accuracy of its test results. The external organizations with which CRHCL is registered for external quality control, are as follows:

1. Clinical Biochemistry: CMC Vellore

2.13. Rejection of Samples:

Samples are rejected in the lab in case of:

1) Incorrect specimen

2) Wrong containers/vacutainers

3) Sample-Requisition form mismatch

4) Leaking containers

5) Inappropriate transport conditions

6) Hemolyzed sample

7) Clotted samples

8) Insufficient sample

In case of a sample rejection, the nursing staff of wards, and OP sample collection center staff are informed immediately, and a prompt attempt is made to collect a repeat sample for analysis.

2.14. Requests for Additional Testing:

CRHCL entertains verbal requests for additional testing in a primary sample already sent to lab. If on sending a specimen for testing and further additional testing is required, please contact the appropriate section of the Laboratory to investigate the feasibility of using the primary specimen for analysis as age of specimen may have impact on the validity of test results. Ideally, a request form should accompany a additional request but, the lack of the request form should not impede the processing of an urgent request. Verbal request for additional tests should be accompanied by a written request later before the dispatch of reports.

2.15. Reporting the Results:

Printed reports are issued to outpatients on submitting the bill. Reports to the inpatients are collected by the respective ward attendants/or patients relatives. Request for reports by telephone are not entertained. For inpatient wards, provisional reports are available once the results are out. Final reports are available only after verification by authorized signatories of lab. It is the policy of the Laboratory to telephone reports only when results for specific clinical parameters have reached critical levels and there is urgency for primary management. Request for verbal reports are not entertained except in urgent cases.

2.16. Policy for remittance of charges in case of non-testing:

In case of non-testing of already collected specimen due to any reason, CRH credits the already paid charges to the customer.

2.17. Sending specimen from outside hospitals/ labs to CRHCL:

Packing procedure for the transport of diagnostic specimens Specimen to be sent should be collected in a suitable primary container (vacutainers for blood samples; sterile plastic bottles for urine, body fluids, sputum and semen samples; sterile plastic container for stool samples for microbiology tests). Please refer to the DOS & Primary sample collection manual provided by CRHCL, for details of containers and type of samples to be collected for each test done in CRHCL. The referring labs/hospitals could get the containers issued from CRHCL or could use the appropriate containers as specified above. Ensure that all specimen container caps/lids are properly tightened to prevent leakage. Label the container properly with patient name, age, gender and patient ID No. from the hospital/lab which is sending sample.

1. Wrap the container in tissue paper or cotton wool, which will act as absorbent material in event of any spillages.

2. This will be placed in a plastic bag/pouch. In case of transport from those places requiring more than two hours to reach CRHCL, Pre frozen gel pack (ice pack) should be placed along with specimen. This is applicable to test requests for biochemistry, microbiology, body fluids (cell count, cell type and cytology) , and clinical pathology (urine samples). Between specimen container and gel pack place a sponge to avoid any damage to specimen container.

Please note: It is preferable to centrifuge the blood samples (in case of tests requiring serum or plasma) for transports requiring more than 2 hours, in order to avoid haemolysis. For arterial blood gas analysis, the maximum time allowed (from sample collection)for the sample to reach CRHCL is 15 minutes, and the sample should be sent in ice box.

3. Properly fill the requisition form.

4. Mention the name, address and contact number of the destination laboratory/Hospital

5. The specimen can be transported manually to CRHCL.

3. General Instructions on Patient Preparation and Sample Collection

3.1. GENERAL INSTRUCTIONS ON PATIENT PREPARATION:

- Patient is informed about the nature of sample to be collected and the tests to be carried out.
- Patient should not have undergone vigorous exercise prior to the test.
- It is necessary to find out if the patient is under any medication.
- The day before and also on the day of test, the patient should not consume Intoxicating substances.
- Do not collect the sample when the patient is under emotional stress, make him/her comfortable.

3.2. PRECAUTIONS TO BE TAKEN BY THE PERSONNEL COLLECTING THE SAMPLE:

1) Fill the particulars of the patient in the requisition form.

2) Label the containers appropriately before collecting the sample.

3) Collect in appropriate containers.

4) Collect appropriate volume

5) Collect at appropriate time

6) Use proper collection technique

7) Use appropriate Tube draw or syringe draw order (Preferably Tube draw order)

3.3. SAMPLE COLLECTION TECHNIQUE: ROUTINE VENIPUNCTURE GUIDELINES:

3.3.1. MATERIALS

1) Syringes

2) Blood Collection Tubes. The vacuum tubes are designed to draw a predetermined volume of blood. Tubes with different additives are used for collecting blood specimens for specific types of tests. The color of the rubber stopper is used to identify these additives. See Selecting the Appropriate Collection Tube and Specimen Container Types.

3) Tourniquets. Latex-free tourniquets are available

4) Antiseptic.

5) cotton.

6) Sharps Disposal Container. A puncture proof container marked "Biohazardous".

3.3.2. SAFETY

1) Observe universal (standard) safety precautions. Observe all applicable isolation procedures.

2) PPE's should be worn at all time while collecting samples.

3) Gloves are to be worn during all phlebotomies .Gloves are changed for every 10 patients‟ phlebotomy, and also in case of any sample spillage over gloves, obvious skin infections in the patient or if the gloves are damaged. After each patient sample collection, worn gloves are cleaned by rubbing with chlorhexidine gluconate hand washing product (recommended by Hospital Infection Control Committee). Palpation of phlebotomy site may be performed without gloves providing the skin is not broken.

4) A lab coat or gown must be worn during blood collection procedures.

5) Needles and hubs are single use and are disposed of in an appropriate 'sharps' container as one unit.

6) All items used for the procedure must be disposed of according to proper biohazardous waste disposal policy.

7) Contaminated surfaces must be cleaned with freshly prepared 1% hypochlorite solution. All surfaces are cleaned daily with antiseptic solutions.

8) In the case of an accidental needle stick, immediately wash the area with an antibacterial soap, express blood from the wound, and contact your supervisor.

3.3.3. PROCEDURE FOR VENEPUNCTURE:

1) Identify the patient. Outpatients are called into the phlebotomy area and asked their name, and age. This information must match the requisition.

2) Reassure the patient that the minimum amount of blood required for testing will be drawn.

3) Assemble the necessary equipment appropriate to the patient's physical characteristics.

4) Wash hands and put on gloves.

5) Position the patient with the arm extended to form a straight-line form shoulder to wrist.

6) Do not attempt a venepuncture more than twice. Notify your supervisor or patient's physician if unsuccessful.

7) Select the appropriate vein for venepuncture. The larger median cubital, basilar and cephalic veins are most frequently used, but other may be necessary. Veins will become more prominent if the patient closes his fist tightly.

3.3.4. Factors to consider in site selection:

- Extensive scarring or healed burn areas should be avoided
- Specimens should not be obtained from the arm on the same side as a mastectomy.
- Avoid areas of hematoma.
- If an IV is in place, samples may be obtained below but NEVER above the IV site.
- Do not obtain specimens from an arm having a cannula, fistula, or vascular graft.
- Allow 10-15 minutes after a transfusion is completed before obtaining a blood sample.

3.3.5. Apply the tourniquet 3-4 inches above the collection site. Never leave the tourniquet on for over 1 minute. If a tourniquet is used for preliminary vein selection, release it and reapply after two minutes.

Clean the puncture site by making a smooth circular pass over the site with the 70% isopropyl alcohol, moving in an outward spiral from the zone of penetration. Allow the skin to dry before proceeding. Do not touch the puncture site after cleaning.

3.3.6. Perform the venepuncture as follows ----------

1) Attach the appropriate needle to the hub by removing the plastic cap over the small end of the needle and inserting into the hub, twisting it tight.

2) Remove plastic cap over needle and hold bevel up.

3) Pull the skin tight with your thumb or index finger just below the puncture site.

4) Holding the needle in line with the vein, use a quick, small thrust to penetrate the skin and enter the vein in one smooth motion.

5) Holding the hub securely, insert the first vacutainer tube following proper order of draw into the large end of the hub penetrating the stopper. Blood should flow into the evacuated tube.

6) After blood starts to flow, release the tourniquet and ask the patient to open his or her hand.

7) When blood flow stops, remove the tube by holding the hub securely and pulling the tube off the needle. If multiple tubes are needed, the proper order of draw to avoid cross contamination and erroneous results is as follows:

Closure color	Collection Tube / Vacuum Tube	Investigations	Mix by inverting
Light Yellow	Blood Culture-SPS	Blood Culture	8-10 times
Light Blue	Citrate Tube	Coagulation Parameters	3-4 times
Dark Yellow	SST-Gel separating Tube	Serum analyte measurement	5 times
Red	Serum Tube	Serum analyte measurement	5 times
Dark Green	Heparin Tube	Plasma analyte measurement	8-10 times
Lavender	EDTA Tube	Plasma analyte measurement	8-10 times
Grey	Fluoride Tube	Plasma analyte measurement	8-10 times

8) Each coagulation tube (light blue top) should be gently inverted 4 times after being removed from the hub. Red and yellow tops should be inverted 5 times. All other tubes containing an additive should be gently inverted 8-10 times. DO NOT SHAKE OR MIX VIGOROUSLY.

9) Place a gauze pad/cotton ball over the puncture site and remove the needle.
10) Immediately apply slight pressure. Ask the patient to apply pressure for at least 2 minutes. 11) When bleeding stops, apply a fresh bandage, gauze or tap
12) Properly dispose of hub with needle attached into a sharps container. Label all tubes with patient labels, initials, date and time.

3.3.7. Infant/Child Phlebotomy:

Choice of procedure and site: The choice of site and procedure (venous site, finger-prick or heel-prick – also referred to as "capillary sampling" or "skin puncture") will depend on the volume of blood needed for the procedure and the type of laboratory test to be done. Venepuncture is the method of choice for blood sampling in term neonates; however, it requires an experienced and trained phlebotomist. If a trained phlebotomist is not available, the physician may need to draw the specimen. The blood from a capillary specimen is similar to an arterial specimen in oxygen content, and is suitable for only a limited number of tests because of its higher likelihood of contamination with skin flora and smaller total volume.

Finger and heel-prick: Whether to select a finger-prick or a heel-prick will depend on the age and weight of the child. Patient immobilization is crucial to the safety of the paediatric and neonatal patient undergoing phlebotomy, and to the success of the procedure. A helper is essential for properly immobilizing the patient for venepuncture or finger-prick. Venepuncture is the preferred method of blood sampling for term neonates, and causes less pain than heel-pricks

Conditions influencing the choice of heel or finger-prick:

Condition	Heel-Prick	Finger-Prick
Age	Birth to about 6 months	Over 6 months
Weight	From 3–10 kg, approximately	Greater than 10 kg
Placement of Lancet	On the medial or lateral plantar surface	On the side of the ball of the finger perpendicular to the lines of the fingerprint
Recommended Finger	Not applicable	Second and third finger (i.e. middle and ring finger); avoid the thumb and index finger because of calluses, and avoid the little finger because the tissue is thin

- Using the guidance given in the table, decide whether to use a finger or heel-prick, and decide on an appropriate size of lancet.
- DO NOT use a surgical blade to perform a skin puncture.
- DO NOT puncture the skin more than once with the same lancet, or use a single puncture site more than once, because this can lead to bacterial contamination and infection.

Prepare the skin :

- Prepare the skin as described above for adult patients.
- DO NOT use povidone iodine for a capillary skin puncture in paediatric and neonatal patients; instead, use alcohol, as stated in the instructions for adults.

Puncture the skin:

- Puncture the skin as described above for adult patients.
- If necessary, take the following steps to improve the ease of obtaining blood by finger-prick in paediatric and neonatal patients: ask the parent to rhythmically tighten and release the child's wrist, to ensure that there is sufficient flow of blood; keep the child warm by removing as few clothes as possible, swaddling an infant in a blanket, and having a mother or caregiver hold an infant, leaving only the extremity of the site of capillary sampling exposed.
- Avoid excessive massaging or squeezing of fingers because this will cause haemolysis and impede blood flow.

3.3.8. Please see the below example for explaining the phlebotomy procedure to a patient

Explaining the procedure to a patient

I will introduce a small needle into your vein and gently draw some blood for _____ tests. (Tell the patient the specific tests to be done).

The results will be made available to your after ----------time (turnaround time), and you need to produce the bill while receiving the reports.

3.4. Collection of Blood Sample from Central Line (Central Venous Catheter, CVC blood Collection)

3.4.1. Indications for CVC blood collection:

CVCs should not be routinely accessed for blood collection. In most cases it is preferable to obtain blood samples from a peripheral vein. If it is known that a venepuncture for blood specimen collection is difficult or not possible, a CVC may be used. Practitioners need to be aware that some CVC blood results (e.g. drug levels, electrolytes, coagulation studies) may be altered depending on the intravenous infusion or locking solution infused in the CVC. Careful attention to the technique of obtaining blood from the CVC must be observed. If laboratory values appear inaccurate, redraw a blood sample from a peripheral vein. Requisitions and tubes should be labelled “line draw”, which will assist to informing clinicians to use caution when interpreting results.

3.4.2. Procedure:

1) Check patient care orders for lab tests required. Ensure the order of draw, number of times specimen tube needs inversion, and the technical factors (eg. patient fasting, specimen on ice, timeliness of dispatch to laboratory) are known and followed prior to obtaining specimen.

2) Perform hand hygiene.

3) Assemble equipment.

4) Identify patient using two identifiers and explain procedure.

5) If continuous infusion of IV fluids in any lumen STOP all IV solutions for 1-3 minutes prior to draw.

6) Perform hand hygiene. Apply protective gloves.

7) Disconnect the tubing from the injection cap.

8) Scrub the Hub with alcohol for 30 seconds (twist back and forth). Completely air dry 30 seconds.

9) Attach prefilled normal saline syringe into injection cap. • Slowly flush saline into catheter catheter using turbulent (push/pause) flushing method

10) After flushing the catheter, withdraw fluid until blood is visualized in the syringe. Slowly withdraw an additional 2ml (minimum waste) and discard.

• Exceptions for NICU patients: Withdraw 1-2 ml of blood if needed, return to patient according to unit procedure.

11) Connect the vacutainer sleeve onto the injection cap.

12) Attach Vacutainer tubes and fill with blood.

13) Follow the order of tubes for multi- specimen draw.

14) Mix the samples by inversion of vacuum tubes.

15) Follow the labeling and transport instructions as already explained for venipuncture.

16) Flush the line using a push/pause flushing method. Wash out any infusate or blood from the catheter.

17) Repeat if necessary to clear all remaining blood from the catheter. Maintain positive pressure, clamp the tubing and disconnect the syringe . Restart the infusion.

3.5. TROUBLESHOOTING HINTS FOR BLOOD COLLECTION:

If a blood sample is not attainable:

- Reposition the needle.
- Ensure that the collection tube is completely pushed onto the back of the needle in the hub.
- Use another tube as vacuum may have been lost.
- Loosen the tourniquet.
- Probing is not recommended. In most cases, another puncture in a site below the first site is advised.

- A patient should never be stuck more than twice unsuccessfully by a phlebotomist. The Supervisor should be called to assess the patient.

3.6. Collection of Arterial Blood :

Sites: Preferred Radial artery. Alternate sites are brachial artery or femoral artery.

Alternate sites have disadvantages: may be harder to locate, because they are less superficial than the radial artery; have poor collateral circulation; are surrounded by structures that could be damaged by faulty technique.

Inappropriate collection and handling of arterial blood specimens can produce incorrect results. Reasons for an inaccurate blood result include:

- presence of air in the sample;
- collection of venous rather than arterial blood;
- an improper quantity of heparin in the syringe, or improper mixing after blood is drawn; a delay in specimen transportation.

Equipment and supplies : Assemble the relevant items (as for venepuncture); plus the following specimen collection equipment and supplies:

- pre-heparinized syringe;
- needles (20, 23 and 25 gauge, of different lengths) – choose a size that is appropriate for the site (smaller gauges are more likely to lyse the specimen);
- a bandage to cover the puncture site after collection;
- a container with crushed ice for transportation of the sample to the laboratory (if the analysis is not done at the point of care);
- where applicable, local anaesthetic and an additional single-use sterile syringe and needle.

Procedure for arterial blood sampling using radial artery:

For sampling from the radial artery using a needle and syringe, follow the steps outlined below.

1. Approach the patient, introduce yourself and identify the patient properly.

2. Place the patient on their back, lying flat. Ask the nurse for assistance if the patient‟s position needs to be altered to make them more comfortable. If the patient is clenching their fist, holding their breath or crying, this can change breathing and thus alter the test result.

3. Locate the radial artery by performing an Allen test for collateral circulation. If the initial test fails to locate the radial artery, repeat the test on the other hand. (A modified Allen test measures arterial competency, and should be performed before taking an arterial sample. Instruct the patient to clench his or her fist; if the patient is unable to do this, close the person‟s hand tightly. Using your fingers, apply occlusive pressure to both the ulnar and radial arteries, to obstruct blood flow to the hand. While applying occlusive pressure to both arteries, have the patient relax his or her hand, and check whether the palm and fingers have blanched. If this is not the case, you have not completely occluded the arteries with your fingers) . Once a site is identified, note anatomic landmarks to be able to find the site again. If it will be necessary to palpate the site again, put on sterile gloves.

4. Perform hand hygiene, clear off a bedside work area and prepare supplies. Put on an impervious gown or apron, and face protection, if exposure to blood is anticipated.

5. Disinfect the sampling site on the patient with 70% alcohol and allow it to dry.

6. If the needle and syringe are not preassembled, assemble the needle and heparinized syringe and pull the syringe plunger to the required fill level recommended by the local laboratory.

7. Holding the syringe and needle like a dart, use the index finger to locate the pulse again, inform the patient that the skin is about to be pierced then insert the needle at a 45 degree angle, approximately 1 cm distal to (i.e. away from) the index finger, to avoid contaminating the area where the needle enters the skin.

8. Advance the needle into the radial artery until a blood flashback appears, then allow the syringe to fill to the appropriate level. DO NOT pull back the syringe plunger.

9. Withdraw the needle and syringe; place a clean, dry piece of gauze or cotton wool over the site and have the patient or an assistant apply firm pressure for sufficient time to stop the bleeding. Check whether bleeding has stopped after 2–3 minutes. Five minutes or more may be needed for patients who have high blood pressure or a bleeding disorder, or are taking anticoagulants.

10. Remove the needle; remove air bubble if any by pressing the plunger of syringe.

11. put the black cap. Mix the blood by gentle inversion 6-8 times.

12. Label the syringe; Place the syringe in an ice box, and send to the lab immediately.

13. Dispose appropriately of all used material and personal protective equipment. . Remove gloves and wash hands thoroughly with soap and water, then dry using single-use towels; alternatively, use alcohol rub solution.

14. Check the patient site for bleeding. If required, use additional pressure for stopping bleeding.

3.7. INTERFERING FACTORS: Reasons for deviations may include-

1. Incorrect specimen collection, handling, storage or labelling.

2. Wrong preservatives or lack of preservatives.

3. Delayed specimen delivery.

4. Incorrect or incomplete patient preparation

5. Hemolyzed blood samples

6. Incomplete sample collection, especially of timed samples.

7. Old or deteriorating specimens

8. Incorrect pretest diet

9. Current drug therapy

10. Type of illness

11. Dehydration

12. Position /activity at time of specimen collection

13. Postprandial status

14. Time of the day

15. Pregnancy

16. No adherence or noncompliance with instructions and pretest preparation

17. Age and gender.

18. Undisclosed drug or alcohol use.

3.8. CSF COLLECTION:

CSF samples are collected by physicians and sent manually to the lab.

PROCEDURE

- Place the patient in a side-lying position with the head flexed onto the chest and knees drawn up to, but not compressing, the abdomen to allow bending the back. This position helps to increase the space between the lower lumbar vertebrae so that the spinal needle can be inserted more easily between the spinal processes. However, a sitting position with the head flexed to the chest can be used. The patient is helped to relax and instructed to breathe slowly and deeply with his or her mouth open.
- Select the puncture site, usually between L4 and L5 or lower. There is a small bony landmark at the L5-S1 interspace known as the surgeon's delight.• that helps to locate the puncture site. The site is thoroughly cleansed with an antiseptic solution, and the surrounding area is draped with sterile towels in such a way that the drapes do not obscure important landmarks.
- Inject a local anaesthetic slowly into the dermis around the intended puncture site.
- Insert a spinal needle with stylet into the midline between the spines of the lumbar space and slowly advance until it enters the subarachnoid space. The patient may feel the entry as a pop of the needle through the Dura mater. Once this happens, the patient can be helped to straighten his or her legs slowly to relieve abdominal compression.
- Remove the stylet with the needle remaining in the subarachnoid space, and attach a pressure manometer to the needle to record the opening CSF pressure.
- Take up to 3 samples of 2 to 3 mL each, place in separate sterile vials, and label sequentially. One tube is used for biochemistry; one is used for microbiology and serology studies; and one is used for haematology cell counts.

Pre-test Patient Care :

Explain the purpose, benefits, and risks of lumbar puncture and explain tests to be performed on the CSF specimen; present a step-by-step description of the actual procedure. Emphasize the need for patient cooperation. Assess for contraindications or impediments such as arthritis. Sedation or analgesia may be used.

- Help the patient to relax by having him or her breathe slowly and deeply. The patient must refrain from breath holding, straining, moving, and talking during the procedure.
- Follow guidelines in Chapter 1 regarding safe, effective, informed pre-test care.

Post test Patient Care :

Have the patient lie prone (flat or horizontal, or on the abdomen) for approximately 4 to 8 hours. Turning from side to side is permitted as long as the body is kept in a horizontal position.

- Women may have difficulty voiding in this position. The use of a fracture bedpan may help.
- Fluids are encouraged to help prevent or relieve headache, which is a possible result of lumbar puncture.
- Interpret test outcomes. Assess and monitor for abnormal outcomes and complications such as paralysis (or progression of paralysis, as with spinal tumor), hematoma, meningitis,
- Initiate infection control precautions if test outcomes reveal an infectious process.
- Observe for neurologic changes such as altered level of consciousness, change of pupils, change in temperature, increased blood pressure, irritability, and numbness and tingling sensations, especially in the lower extremities.
- If headache occurs, administer analgesics as ordered and encourage a longer period of prone bed rest
- Check the puncture site for leakage.
- Document the procedure completion and any problems encountered or complaints voiced by the patient.

Clinical Alert

- Extreme caution should be used when performing lumbar puncture:
- If ICP is elevated, especially in the presence of papilledema or split cranial sutures. However, with some cases of increased ICP, such as with a coma, intracranial bleeding, or suspected meningitis, the need to establish a diagnosis is absolutely essential and outweighs the risks of the procedure.
- A relative contraindication would be raised ICP from a suspected mass lesion. To reduce the risk for brain herniation, a less invasive procedure such as a CT scan or magnetic resonance imaging (MRI) should be done.

Contraindications to lumbar puncture include the following conditions:

- Suspected epidural infection
- Infection or severe dermatologic disease in the lumbar area, which may be introduced into the spinal canal
- Severe psychiatric or neurotic problems
- Chronic back pain
- Anatomic malformations, scarring in puncture site areas, or previous spinal surgery at the site
- If there is CSF leakage at the puncture site, notify the physician immediately and document findings.

3.9. PATIENT CHECKLIST FOR SELF-MONITORING OF BLOOD GLUCOSE (SMBG) TESTING:

This list is a general outline. Each brand of meter has its own instructions. Read the instructions on each new meter carefully to get accurate results. Know whether the monitor and strips give whole blood or plasma equivalent results.

General instructions:

- Make sure hands are clean, dry, and warm.
- Prick finger with the lancet.
- Squeeze out a drop of capillary blood.
- Drop the blood onto the test strip or sensor.
- Wait for the test strip or sensor to develop.
- Compare the test strip to the chart or insert it in the meter.
- Safely dispose of lancet in an approved sharps container.
- Record blood glucose results with date and time.

3.10. ORAL GLUCOSE TOLERANCE TEST:

TYPES OF OGTT-

1. Children -2 hours OGTT (fasting sample & post glucose load 2 samples -1 hour, 2 hour after 1.75gm/kg body weight glucose load).

2. Adults - 2 hours OGTT (fasting sample & post glucose load 2 samples -1 hour, 2 hour after 75 gm glucose load).

3. Pregnant women - 3 hours OGTT (fasting sample & post glucose load 3 samples-1 hour, 2 hour ,3 hour after 100gm glucose load).

Patient Preparation

1. Patient should be in normal carbohydrate diet for last 3 days.

2. For 3 days prior to the test, medications known to affect glucose utilization should be discontinued when possible.

3. Strenuous exercise on the previous day and on same day should be avoided.

4. Patient should be in fasting for 12 hours.

5. Patient should not smoke and drink alcohol on the same day or previous day.

6. Patient should be seated in comfortable position.

Glucose Load : For Adults (Male and Female): 75 gram anhydrous glucose in 250-300 ml of water. (For 2 hour GTT) Children: 1.75g/kg of body weight not exceeding 75 gram (For 2 hour GTT). Pregnant women: 100 gram of glucose in 250-300 ml of water (For 3 hour GTT).

Note: Ask the patient to drink glucose solution slowly within period of 5 min to avoid vomiting. If patient vomits, test should NOT be continued, and ask the patient to come again next day.

Sample collection:

FOR 2 HOURS GTT-

- After 10-12 hours of fasting, blood sample is collected in the morning before the glucose load.
- Then glucose load is given orally. Blood sample is collected every hour for 2 hours post glucose load.

FOR 3 HOURS GTT-

- After 10-12 hours of fasting, blood sample is collected in the morning before the glucose load.
- Then glucose load is given orally. Blood sample is collected every hour for 3 hours post glucose load.

3.11. Instructions for collection of Urine (Random/Spot)

The patient should use a sterile, wide-mouthed leak-proof container for urine collection. The container will be provided in the hospital. Urinate the first 10 ml into the toilet. Collect 10 ml voided urine in the container by moving into the stream of urine without halting or restarting the stream. Close and secure the lid of the container to make sure that there is no leakage.

3.12. INSTRUCTIONS FOR 24 HOUR URINE COLLECTION AT HOME (For OP) : Please collect the 24 hour urine sample as per the instructions given ---

1. For some tests, chemical preservatives may be required. .

2. For most of the tests, refrigeration is the best method of preservation.

How to collect ?

- Take a clean and dry plastic jar to collect 24 hr urine sample.
- The urine collection should start in the morning, and continued till next day morning.
- Don't collect the first voided urine of morning , on the starting day.
- Start collecting second voided urine till next day for 24 hours . For example, if you have started collecting urine at 8.30 AM , continue collecting urine till next day 8.30 AM.
- If a chemical preservative has to be used, put the preservative first into the container , and then start collecting urine for next 24 hours .
- If the preservation is by refrigeration, then after collection of urine every time, keep the container in the refrigerator.
- The whole urine sample in the container has to be delivered to the lab.

Note :

1. Don"t spill any urine collected;
3. always keep the container with urine in the refrigerator during the 24 hour collection.

3.13. 24 Hours Urine Collection for IP:

Please collect the 24 hour urine sample as per the instructions given ---

1. For some tests, chemical preservatives may be required..
2. For most of the tests, refrigeration is the best method of preservation.

Sterile plastic can has to be used for urine collection

- The urine collection should start in the morning, and continued till next day morning.
- Don't collect the first voided urine of morning, on the starting day.
- Start collecting second voided urine till next day for 24 hours. For example, if you have started collecting urine at 8.30 AM, continue collecting urine till next day 8.30 AM.
- If a chemical preservative has to be used, put the preservative first into the container, and then start collecting urine for next 24 hours.
- If the preservation is by refrigeration, then after collection of urine every time, keep the container in the refrigerator.
- Measure the 24 hour urine volume. Send 10 ml of this sample to the lab for analysis; inform the 24 hour volume to the lab.

Note: Don't spill any urine collected; Urine sample should be directly voided into the container using a funnel.

3.14. Preservatives for 24 Hour Urine Collection:

Analyte	**Preservative**
Amylase	Refrigeration
Calcium	10 ml of concentrated HCl
Chloride	Refrigeration
Creatinine	Refrigeration
Microalbumin	Refrigeration
Protein	Refrigeration
Potassium	Refrigeration
Phosphorus	10 ml of concentrated HCl
Sodium	Refrigeration
Uric Acid	10 ml of 10% NaOH
Urea	Refrigeration

In case of non-availability of refrigerator for outpatients at home, 10 grams of boric acid can be used as preservative

3.15. Disposal of Biomedical Waste: Waste to be segregated and disposed as per the following guidelines ---

- Non-infective waste materials such as waste papers, plastic covers of syringes, covering box of reagents etc. will be collected in a covered black bucket and will be disposed in the incinerator.
- containers will be cleaned after every use with appropriate disinfectant(s).

*These may undergo changes according to the Hospital Bio-waste Management.
Follow whatever is current guidelines prescribed by them.

YELLOW BAG

- Human Anatomical waste
- Soiled waste
- Expired/discarded medicines
- Chemical waste
- Chemical liquid waste
- Gauze, disposable clothing items contaminated with blood or body fluids

BLUE BAG

GLASSWARE

- Broken/Discarded and contaminated glass INCLUDING
- **Medicine vials** and Laboratory glassware
- **Ampoules**

RED BAG

CONTAMINATED
WASTE RECYCLABLE: WASTE GENERATED
FROM DISPOSABLE ITEMS

- Tubing's
- IV sets
- Syringe without needles
- Vaccutainers
- Gloves
- Contaminated Plastiware generated in the laboratory eg disposable pipette tips, coagulation tubes, serology cards, etc

WHITE BAG

WASTE SHARPS INCLUDING METALS

- Needles
- Syringe with fixed needles
- Needles from needle tip, cutter or burner
- Broken glassware or any other contaminated sharp objects.

Incinerator:

The Hospital has a large incinerator to which all infective wastes will be sent at regular intervals.

Liquids and chemical wastes:

These will be disinfected by chemical treatment using 1 % Sodium Hypochlorite solution and then discarded into the drain/sewer. This process will be carried out every day. Each day a fresh solution of Sodium Hypochlorite will be prepared.

Test tubes and Glassware:

All the test tubes and glassware used in the biochemistry laboratory will be autoclaved on a daily basis.

Sharp instruments:

Sharp instruments such as needles, blades etc. will be immersed in 1% Sodium Hypochlorite solution and then sent for regular waste disposal by land filling.

Disposal of container containing blood and body fluids:

Method of disposal:

- Vials containing blood and body fluids are to be treated with freshly prepared 1% sodium hypochlorite solution and discarded in red plastic bin with proper labelling.

3.16. Monitoring Samples transported from Outside Hospitals/Labs :

The samples transported from outside labs/hospitals are checked for transport conditions, type of containers and matching between request form –container.

3.17. Sample Rejection Criteria :

In OP sample collection area and Lab Reception Counter, OP/IP samples are rejected based on following criteria :

1) Leaking containers

2) Mismatch of sample container-requisition form /letter

3) Not brought under appropriate transport conditions

4) Wrong containers /vacutainers used

5) Hemolysis

SAMPLE COLLECTION AND TRANSPORT AND STORAGE INSTRUCTIONS. (Storage/Transport conditions: A : Ambient Temperature; R : Refrigeration; F: Frozen)

Test	Sample	Volume of sample to be collected & instruction for collection	Precaution /instruction for transport	Method of Analysis	Turnaround time (TAT)	TAT for urgent tests	Clinical Utility/ Information on the test
ABG (Arterial Blood Gas Analysis)	Heparinised whole blood	2 ml. Blood in pre-Heparinised Syringe. Mix gently. Follow ABG specimen collection instructions.	Transport immediately. Analysis should be done with in 20 min of collection	ISE	Same day after 5 min	-----	Metabolic/ Respiratory Alkalosis/Acidosis
ADA	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum)	R	Spectrophoto-metric	24 Hours	-----	Diagnosis of tubercular meningitis
ADA	CSF	2 ml CSF (without preservative). Follow CSF specimen collection instructions.	R	Spectrophoto-metric	24 hrs	-----	Diagnosis of tubercular effusions
ADA	Other Body Fluids : Ascitic, pleural, periton	2 ml of Fluid	R	Spectrophoto-metric	24 hrs	-----	Diagnosis of Tubercular effusions.

	eal, pericardial, synovial						
A/G Ratio	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Calculation from albumin and globulin values	Same day after 2 hours	1 hour	Altered in Liver disease, Renal disease, Malnutrition and Malabsorption
Albumin	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto-metric-BCG	Same day after 2 hours	1 hour	Used for determining if patient has liver disease or kidney disease.
Albumin	Fluids (Other than CSF & Urine)	2 ml fluid. No preservative	R	Spectrophoto-metric-BCG	Same day after 2 hours	1 hour	Used to differentiate Exudate from Transudate (Light's criteriae)
Serum-Ascitic Albumin Gradient	Serum, ascitic fluid	3 ml blood, 2 ml fluid	R	Calculation From serum & ascitic fluid levels of albumin	Same day after 2 hours	1 hour	Used to differentiate Exudate from Transudate (Light's criteriae)
Alkaline Phosphatase	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto metric-ALP-AMP	Same day after 2 hours	1 hour	Majority of ALP activity is derived from the liver and bone. Concentrations are increased in patients with biliary

							obstructive disorders, tumor of liver and bone etc.
Amylase	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric-CNP-G3	Same day after 2 hours	1 hour	Diagnosis of Pancreatitis
Amylase	Urine 24 hrs	10 ml 24 hrs urine. Quote total volume.	R	Spectrophotometric-CNP-G3	Same day after 2 hours	-----	Pancreatitis and macroamylassemia
Amylase	Urine Spot	10 ml urine.	R	Spectrophotometric-CNP-G3	Same day after 2 hours	-----	Pancreatitis and macroamylassemia
Amylase	CSF	2 ml fluid	R	Spectrophotometric-CNP-G3	Same day after 2 hours	1 hour	TB diagnosis
Amylase	Other body fluids	2 ml fluid	R	Spectrophotometric-CNP-G3	Same day after 2 hours	-----	Pancreatic ascitis; pancreatic effusions

Anti TPO	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Autoimmune thyroiditis
Beta- HCG	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Pregnancy, Ectopic pregnancy, Choriocarcinoma
Bilirubin Direct (Conjugated)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric -Diazo	Same day after 2 hours	1 hour	Used for patients who show signs of abnormal liver function.
Bilirubin Indirect (unconjugated)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Calculation from total and direct bilirubin	Same day after 2 hours	1 hour	Used for patients who show signs of abnormal liver function., and haemolytic causes of jaundice
Bilirubin Total	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric-Diazo	Same day after 2 hours	1 hour	Used for patients who show signs of abnormal liver function.
Bilirubin : Total, direct and indirect	Body fluids	2 ml fluid	R	Spectrophotometric-Diazo	Same day after 2 hours	1 hour	Used for patients who show signs of abnormal liver function.

BUN (Blood Urea Nitrogen)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Calculation form serum urea level	Same day after 2 hours	1 hour	Kidney function test
Calcium	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto metric-Arsenazo	Same day after 2 hours	1 hour	High level in Hyperparathy roidism, Malignancy, Sarcoidosis. Low levels in hypoalbumin emia, renal insufficiency, hypoparathyr oidism.
Calcium	Urine 24 hours	10 ml concentrated HCl as preservative for 24 hrs urine. (Quote Total volume).send 10 ml urine to the lab.	R	Spectrophoto metric-arsenazo	Same day after 2 hours	-----	Diagnosis of hyper and hypoparathyr oi-dism
Calcium	Urine spot	Acidify the urine with concentrated HCl to adjust pH 3-4. 10 ml urine is required.	R	Spectrophoto metric-arsenazo	Same day after 2 hours	1 hour	Diagnosis of hyper and hypoparathyr oidism
Chloride	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ISE	Same day after 2 hours	1 hour	Electrolyte imbalance

Chloride	CSF	2 ml fluid (Without any preservative)	R. Transport as early as possible to increase the relevance of the report	ISE	Same day after 2 hr.	1 hour	For differential diagnosis of meningitis
Chloride	Urine 24 hrs	10 ml 24 hrs urine. (without any preservative)	R	ISE	Same day after 2 hr.	-----	For differential diagnosis of hyperchloremic metabolic acidosis and hypochloremic metabolic alkalosis
Chloride	Urine spot	10 ml spot urine. (without any preservative)	R	ISE	Same day after 2 hr.	1 hour	For differential diagnosis of hyperchloremic metabolic acidosis and hypochloremic metabolic alkalosis
Chloride	Fluids	2 ml body fluid (Without any preservative)	R	ISE	Same day after 2 hours	-----	Used for differential diagnosis of effusions
Cholesterol-HDL (HDL cholesterol)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum (10-12 hrs fasting required)	R	Spectrophotometric-PVS/PEGME	Same day after 2 hours	-----	Risk stratification of IHD

Cholesterol-LDL (LDL Cholesterol)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum (10-12 hrs fasting required)	R	Spectrophotometric-PVS/PEGME	Same day after 2 hours	-----	Risk stratification of IHD
Cholesterol-Total	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum .(10- 12 hours fasting required)	R	Spectrophotometric, CHOD-PAP	Same day after 2 hours	-----	Increased levels related to increased risk of cardiovascular disease.
Total cholesterol – HDL cholesterol ratio	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum .(10- 12 hours fasting required)	R	Calculation from total cholesterol and HDL cholesterol values	Same day after 2 hours	-----	Increased levels related to increased risk of cardiovascular disease.
CKMB Activity	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum. Avoid collection in plasma tubes.	R. Transport as early as possible to increase the relevance of the report	Immunoinhibition, spectrophoto-metric-CK-HK-G6PDH	Same day after 2 hours	1 hour	Myocardial Infarction
CPK Activity Total	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto-metric-CK-HK-G6PDH	Same day after 2 hours	1 hour	Myocardial, Cerebro-vascular, skeletal muscular disease
Creatinine	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Jaffe‟s Kinetic	Same day after 2 hrs	1 hour	Renal function test

Creatinine	Urine 24 hrs	10 ml of 24 hrs urine. No preservative ,Refrigeration needed. If refrigerator is not available, use 10 ml of concentrated HCl or 10 grams boric acid as preservative. Quote total volume.	R	Jaffe‟s Kinetic	Same day after 2 hrs	-----	Renal function test
Creatinine	Spot Urine	10 ml urine	R	Jaffe‟s Kinetic	Same day after 2 hrs	1 hour	Renal function test; also used for calculation of protein : creatinine ration & calcium creatinine ratio
Creatinine Clearance	Serum & Urine	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum & 10 ml 24 hrs urine. (Quote Total volume)	R	Calculation	Same day after 2 hrs	-----	Renal function test
Fertility Hormones (LH, FSH, Prolactin)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Assessment of infertility
FSH	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Gonadal function test

GGTP (Gamma Glutamyl Transferase)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric-L-gammaglutamyl-3-carboxy-4-nitroanilide to glycylglycine Substrate	Same day after 2 hrs	1 hour	Alcoholic liver disease and cholestasis
Globulin	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Calculation from albumin and total protein	Same day after 2 hrs	1 hour	Increased level related to chronic infections, Hepatic diseases, multiple myeloma
Glucose (Random, Fasting, Post prandial)	Plasma	2ml Blood in Fluoride tube for plasma/ plain / gel tube (Anytime,10-12 hrs of fasting, 2 hrs after food)	R	Spectrophotometric-HK-G6PD.	Same day after 2 hours	1 hour	Diabetes mellitus, impaired glucose tolerance and hypoglycaemic conditions
Glucose	CSF	2 ml Fluid, No preservative	Store in ice. Transport as early as possible to increase the relevance of the report	Spectrophotometric-GOD-POD	Same day after 1 hours	1 hour	Used for differentiating bacterial meningitis from others
Glucose	Fluids other than CSF and Urine	2 ml Fluid, No preservative	Store in ice	Spectrophotometric-GOD-POD	Same day after 2 hours	-----	Used for differential diagnosis of effusions

Glucose Tolerance Test (GTT)	Plasma & Urine	2ml Blood in Fluoride tube for plasma./gel/plain tube. 10 ml urine. (FPG,75 g glucose after 30 min, 1 hr, 1.30 hr, 2 hr) (GTT after GCT-FPG, 100 g glucose, 1 hr, 2 hr, 3 hr)	R	Spectrophotometric-HK-G6PD. For glucose estimation etric- GOD-POD .	Same day after 2 hours of last sample	-----	Diagnosis of Diabetes mellitus, Gestational DM, Renal glycosuria.
Glucose Challenge Test (GCT)	Plasma	2ml Blood in Fluoride tube/gel/plain tube. (50 g glucose, after 1 hr)	R	Spectrophotometric GOD-POD	Same day after 2 hours of last sample	-----	Diagnosis of gestational diabetes mellitus
Glycated Hemoglobin (HbA1C)	Whole Blood	2 ml Whole Blood in EDTA tube.	R	Immunoturbidimetry	Same day after 2 hrs.	-----	Diagnosis and management of diabetes mellitus
K (Potassium)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ISE	Same day after 2 hours	1 hour	Differential diagnosis of hypokalemia and hyperkalemia , metabolic acidosis, metabolic alkalosis
K (Potassium)	Urine 24 hrs	10 ml 24 hrs urine. No preservative. Quote total volume.	R	ISE	Same day after two hrs	1 hour	Differential diagnosis of hypokalemia and hyperkalemia ,

K (Potassium)	Urine Spot	2 ml Urine.	R	ISE	Same day after 2 hrs	1 hour	Differential diagnosis of hypokalemia and hyperkalemia ,
LDH	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto metric, Kinetic method (pyruvate to lactate)	Same day after 2 hours	1 hour	Marker enzyme for cell turnover, cell destruction; increased in haemolytic diseaseas, liver diseases, etc...
LDH	Body fluids (pleura l, periton eal, pericar dial, synovi al, ascitic)	2 ml fluid without preservative	R	Spectrophoto metric, Kinetic method (pyruvate to lactate)	Same day after 2 hours	-----	Differential diagnosis of effusions; marker enzyme for cell turnover and cell destruction.
LFT (total protein, albumin, total bilirubin, direct bilirubin, indirect bilirubin, ALT, AST, ALP)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophoto metric methods	Same day after 2 hours	1 hour	Liver function assessment
LH	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Gonad dysfunction. Assessment of Infertility

Lipase	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric, Kinetic	Same day after 2 hours	1 hour	Diagnosis of pancreatitis
Lipase	Body fluids	2 ml fluid	R	Spectrophotometric, Kinetic	Same day after 2 hours	1 hour	Pancreatic effusions
Lipid profile (fasting)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric, enzymatic methods for TG, total cholesterol, LDL-Cholesterol, HDL cholesterol; calculation of VLDL and Choelsterol-HDL ratio	Same day after 2 hours	-----	Hypolipidemia and Hyperlipidemia.
Lithium	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ISE	Same day after 1hrs	-----	Assessment of Manic Depressive illness, therapeutic drug monitoring, Lithium toxicity
Micro Albumin	Urine, 24 hours	10 ml 24 hours urine. No preservative (Quote 24 hours volume)	R	Immunoturbidimetric	Same day after 2 hours	-----	Diagnosis of Nephropathy

Micro Albumin	Urine spot	10 ml spot urine	R	Immunoturbi dimetric	Same day after 2 hours	----	Diagnosis of Nephropathy
Micro TP (CSF Protein)	CSF	2 ml fluid, No preservative	R. Transport as early as possible to increase the relevance of the report	Spectrophoto metric, pyrogallol red method	Same day after 2 hours	-----	Differential diagnosis of meningitis
Micro protein urine (Urine protein)	Urine 24 hours	10 ml 24 hrs Urine. No preservative. Quote Total volume	R	Spectrophoto metric, pyrogallol red method	Same day after 2 hours	-----	Diagnosis and management of diabetic nephropathy and chronic renal failure
Micro protein Urine (TP Urine)	Urine SPOT	10 ml spot urine	R	Spectrophoto metric, pyrogallol red method	Same day after two hours	-----	Diagnosis and management of diabetic nephropathy and chronic renal failure
Protein creatinine ratio	Urine spot	10 ml spot urine	R	Calculation from protein and creatinine	Same day after 2 hours	-----	Diagnosis and management of diabetic nephropathy and chronic renal failure
Na (Sodium)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ISE	Same day after 2 hours	1 hour	Analysis of Hyper or Hyponatraem ia

Na (Sodium)	Urine 24 hrs	10 ml 24 hrs urine. No preservative. Quote the total volume.	R	ISE	Same day after 2 hours	-----	Analysis of Hyper or Hyponatraemia
Na (Sodium)	Urine Spot	2 ml urine	R	ISE	Same day after 2 hours	1 hour	Analysis of Hyper or Hyponatraemia
Osmolality	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	calculation	Same day after 2hrs	1 hour	Used in investigation of hyponatraemia and identification of an osmolar gap
Osmolality	Urine	Urine spot without preservatives, 10 ml.	R	calculation	Same day after 2 hours	1 hour	Used in investigation of hyponatraemia and identification of an osmolar gap
Phosphorous	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	UV-Spectrophotometric-Molybdate	Same day after 2 hours	1 hour	Renal failure, Hypo & Hyperparathyroidism, rickets.
Phosphorous	Urine 24 hrs	10 ml of concentrated HCl to acidify 24 hours urine. Quote 24 hours volume; send 10 ml urine to the lab	R	UV-Spectrophotometric-Molybdate	Same day after 2 hours	-----	Renal failure, Hypo & Hyperparathyroidsm

Phosphorous	Urine spot	2 ml urine. Acidify with HCl after collection. Ph 3-4.	R	UV-Spectrophotometric-Molybdate	Same day after 2 hours	-----	Renal failure, Hypo & Hyperparathyroidsm
Prolactin	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum ,	R	ELFA	Same day after 5 hours	-----	Pituitary tumors, menstrual irregularities, infertility, impotence and galactorrhea.
Prostate Specific Antigen (P.S.A), total	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	For Benign prostatic hypertrophy, prostate cancer. Avoid test for 7 days after PR examination, USG, UTI.
Protein	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Biuret	Same day after 2 hours	1 hour	Useful in evaluating patient‟s nutritional status, liver disease, renal disease and GI disease.
Protein	Fluids (Other than CSF & Urine) - perioto neal, pleural , ascitic, synovi al.	2 ml fluid. No preservative.	R	Biuret	Same day after 2 hours	-----	Levels increased in inflammatory , neoplastic or traumatic conditions

SGOT/AST	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric-AST-MDH-NAD	Same day after 2 hours	1 hour	Analysis of Liver function
SGPT/ALT	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric-ALT-LDH-NAD	Same day after 2 hours	1 hour	Analysis of Liver function
Thyroid Function Test (T3,T4,TSH)	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Thyroid function assessment
T3	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Thyroid function test
T4	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Thyroid function test
TSH	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Thyroid function test

TSH	Cord blood serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	Thyroid function test
Triglycerides	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum (10-12 hrs fasting required	R	Spectrophotometric-LPL-GK-GPO-POD	Same day after 2 hours	-----	High conc. are associated with pancreatitis and increased risk for cardiovascular disease.
Troponin I	plasma /serum	2 ml Blood in gel separating/plain tube for serum/ EDTA tube for plasma.	R. Transport as early as possible to increase the relevance of the report	Card method, chromatographic lateral flow immunoassay	Same day after 1 hours	1 hour	Diagnosis of MI
Troponin T (Cardiac troponin T/ctnT)	Whole blood	2 ml Blood in EDTA tube	R. Transport as early as possible to increase the relevance of the report	Strip method using monoclonal antibody	Same day after 1 hours	1 hour	Diagnosis of MI
Urea	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric, Kinetic, Urease-GLDH	Same day after 2 hours	1 hour	Kidney function test; Pre renal and post renal conditions of increased blood urea.

Urea	Urine 24 hrs	10 ml 24 hrs Urine. No preservative. Quote total volume.	R	Spectrophotometric, Kinetic, Urease-GLDH	Same day after 2 hours	-----	Kidney function test
Urea	Urine spot	2 ml spot urine.	R	Spectrophotometric, Kinetic, Urease-GLDH	Same day after 2 hours	-----	Kidney function test
Urea Clearance	Urine 24 hrs, Plasma	10 ml 24 hrs Urine. No preservative. 2 ml EDTA Plasma.	R	calculation	Same day after 2 hours		Kidney function test
Uric Acid	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	Spectrophotometric, Uricase POD	Same day after 2 hours	1 hour	Gout, disorders of purine metabolism. Lesch-Nyhan Fabconi syndrome
Uric Acid	Urine 24 hrs	10 ml 24 hrs urine. Do not refrigerate. NaOH to alkalinise urine. Ph > 8. Quote total volume.	R	Spectrophotometric, Uricase POD	Same day after 2 hours	----	Gout, disorders of purine metabolism. Lesch-Nyhan & Fanconi syndrome.
Uric Acid	Urine spot	2 ml urine. Alkalinise with 10 ml. 10% NaOH. pH should be > 8.	R	Spectrophotometric, Uricase POD	Same day after 2 hours	-----	Gout, disorders of purine metabolism. Lesch-Nyhan & Fanconi syndrome.

Vitamin D, 25-hydroxy	Serum	3 ml Blood in Plain tube or 3.5 ml Blood in Gel separating tube for serum	R	ELFA	Same day after 5 hours	-----	To diagnose and manage vitamin D deficiency

3.18. In house Transport of Samples from Inpatient Wards /OP sample collection Area to Lab:

1) Specimen containers must be placed in the appropriate boxes (plastic boxes) provided along with request forms

2) Leaking containers/containers that are externally contaminated should not be sent to the laboratory

3) Specimen containers along with request forms are transported manually from the sample collection area to the respective laboratories.

4) In patient samples are sent manually by ward attendants to the sample reception counter.

3.19. Sample Rejection Criteria in CRHCL & Actions to be taken (Once the sample reaches lab sections):

3.20. For sending samples to referral labs:

CRHCL uses logistic services provided by the referral labs. Outsourcing is done through the lab staff who has been given the responsibility for receipt of the samples to be outsourced. The staff send the samples after considering the MOU and list of tests, as per review done by lab management.

4. Specific Information On Biochemistry

A : Ambient Temperature; R : Refrigeration; F: Frozen

5. Specific Information on Microbiology:

The Gram Stain

Intended use: The Gram Stain Reagents and Kit are recommended for the differential staining of bacteria in primary specimens and from culture.

SUMMARY AND EXPLANATION OF THE TEST The Gram stain is well established as an important aid in the differentiation and identification of isolated microorganisms. A correctly performed and interpreted Gram stain of certain clinical specimens also can be a rapid source of presumptive diagnostic information. Numerous studies and reports have established the value and importance of this technique in the examination of sputum and transtracheal specimens, cerebrospinal, and other normally sterile body fluids, uncontaminated abscess fluids and specimens from soft tissue infections, and exudates from the male urethra. The Gram stain examination of uncentrifuged urine also is a procedure recommended to provide rapid preliminary information. Gram stain examinations should not be used, however, as a substitute for complete and careful culture studies.

PRINCIPLES OF THE PROCEDURE: The Gram stain serves to divide bacteria into two main groups: gram positive organisms that retain the primary crystal violet dye and appear deep blue or purple, and gram negative organisms which can be decolorized easily, take up the counterstain safranin and appear red or pink. Gram positive organisms have increased cross linked teichoic acids and a lower lipid content in their cell wall that cause decreased permeability to organic solvents and they retain the crystal violet stain. Gram negative organisms have a higher lipid content in their cell wall, which increases permeability to decolorizer so they lose the crystal violet and take up the counterstain.

REAGENTS/MATERIALS:

1. Gram Crystal Violet Solution, BD a. Approximately 0.4% crystal violet in an aqueous alcohol solution. 2. Gram Iodine Solution (Stabilized), BD a. Approximately 13% polyvinylpyrrolidone-iodine complex in 1.9% aqueous potassium iodine

3. Gram Decolorizer Solution, BD a. Denatured ethyl alcohol and acetone, approximately three parts to one part, respectively

4. Gram Safranin Solution, BD a. Approximately 0.25% safranin in 20% ethyl alcohol

PRECAUTIONS: For invitro diagnostic use.

As with all techniques involving pathogenic and potentially pathogenic microorganisms, established aseptic practices should be consistently applied throughout his procedure.

These reagents are harmful or fatal if swallowed and can cause eye irritation if contact is made.

In event of eye contact, flush eyes with an eye wash system or tap water for 15 minutes.

Gram decolorizer solution is flammable and its vapors; may be harmful; use in a well-ventilated area away from open flames.

SPECIMEN COLLECTION AND PREPARATION:

The Gram stain may be performed on smears prepared from clinical specimens or samples containing mixed flora or pure cultures or on smears of microbial growth from laboratory cultures.

QUALITY CONTROL: As a test of both reagent integrity and correct reading and staining technique, the daily (or with each new shipment of stain) performance of quality controls is required. This is especially important when clinical specimens are being examined to provide presumptive diagnostic information or a guide for antimicrobial therapy. Overnight (18-24 hour) cultures of Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive) are suitable control organisms. More subtle deficiencies in reagent quality and techniques can be detected by the use of weakly reactive bacteria such as Bacillus subtilis (Gram positive) and Moraxella catarrhalis (Gram negative).

PROCEDURE:

METHANOL FIXED GRAM STAIN NOTE: Methanol fixation preserves the morphology of red blood cells as well as bacteria and is especially useful for examining blood specimens and blood cultures.

1. Flood slide with crystal violet for 5 seconds.

2. Rinse gently with tap water.

3. Flood with Gram's iodine for 10 seconds.

4. Rinse gently with tap water.

5. Decolorize with alcohol-acetone decolorizer for 5 seconds or until blue color stops running.

6. Rinse gently with tap water.

7. Flood with safranin counterstain for 5 seconds.

8. Rinse, air dry and examine. P

ROCEDURAL NOTES: Gram stain slides may be decolorized and restained if necessary. Remove oil with KimTech wipes, flood smear with decolorizer until smear appears colorless, and then re-stain.

INTERPRETATION: When the differential Gram procedure is performed correctly, organisms which retain the primary stain mordant complex will appear microscopically blue to purple and

are termed “Gram-positive”; organisms which are decolorized and therefore take up the counterstain microscopically will appear pink to red and are termed “Gram-negative”.

MORPHOLOGY:

1. Rods: Elongated organisms, longer than wide

2. Cocci: Round organisms, may be in singles, pairs, chains or clusters.

3. Yeast: Organisms that may be oval in shape, longer than bacteria, may exhibit “budding” or pseudohyphae.

4. Coccobacillus: Organisms that appear somewhat elongated, but not enough to call bacillus.

Sputum:

- Gram stains should be examined as soon as possible to determine the quality of the specimen.
- Examine at least 10 fields under low power (10X) to determine the number of WBC’s and epithelial cells present.
- Report per low power field as >25 or 10 per oil immersion field)

Synovial fluid Gram stains.

- Report crystals if present. Also comment on their shape, either “needle” or “rhomboid.”
- Extra care should be taken when staining this type of fluid as this fluid type is prone to stain precipitate formation. It is recommended that these are hand stained.
- CSF Gram stains: CSF Gram stains should be resulted as soon as possible, especially those on in-house patients.

LIMITATIONS OF THE PROCEDURE:

1. Gram reactivity is not an absolute characteristic and is influenced by several factors. The age of culture affects its degree of Gram positivity. It is recommended that young, actively growing cultures be used for gram staining.

2. An intact cell wall is required for an accurate gram stain. Older cultures may have breaks in the cell wall and may give a gram variable result. Although the optimum age for staining may vary from species to species, it is general practice to examine 18-24 hour cultures. When experiences or special conditions dictate, younger or older cultures also should be tested.

3. Different species of bacteria may not be equally sensitive to the deleterious effects of heat fixation, but overheating, because it may cause gram positive bacteria to stain gram negative, should be avoided.

4. Antimicrobial agents also may make gram positive organisms more susceptible to decolorization; this fact should be kept in mind when examining clinical specimens, especially from treated patients.

5. The Gram stain should be used only to provide supplementary diagnostic or taxonomic information; it is not intended, and should not be used, as a substitute for more comprehensive tests.

ZIEHL NEELSEN (ZN) STAINING

PRINCIPLE: The property of acid-fastness of Mycobacteria is based on the presence of Mycolic acid in their cell wall. Primary stain (fuchsin) binds to cell wall Mycolic acids. Intense decolourization (strong acid) does not release primary stain from the cell wall and AFB retain the red colour of fuchsin. Counter stain (Methylene blue) provides contrasting background.

MATERIALS REQUIRED:

• Sputum container to collect sputum.

• Sterile 1 oz. universal containers with identification number engraved cap.

• Wire loop with an inner diameter of 5 mm to spread sputum on the slide

• Clean new, washed microscopy slide (no grease and no scratches on the slide)

• Diamond marker to enter identification number on the microscopy slide

• Forceps to hold slide with sputum smear

• Bunsen burner to fix smear

• Metal waste bin with disinfectant (5% phenol solution) to discard infected material

• Staining rack to hold the slides

• Slide rack to place stained smear slides to dry in the air

• 1% Carbol-fuchsin

• 25% H_2SO_4

• 0. 1% Methylene blue

• Tap Water

Collection of Sputum collection, selection of the purulent portion for smear preparation and making smear is critical for good quality of smears.

Size: Take purulent portion of sputum and prepare 2 - 3 cm length X 1 - 2 cm wide or 3 X 2 cm (100-150 fields to be counted in one length) smear in the center of the slide.

Evenness: Firmly make smear perpendicular to the slide (move in small concentric circles or coil like patterns).

Thickness: Place the slides on the piece of printed-paper. If letters cannot read it is too thick. Allow the smear to air dry completely at room temperature. After air drying, fix the slide by passing it on the flame 3-4 times

STAINING PROCEDURE

• Place the slides on a staining rack in batches (maximum 12) with the smeared side facing up. Ensure that the slides do not touch each other

• Flood entire slide with filtered 1 % Carbol-fuchsin.

• Heat each slide slowly until it is steaming. Do not boil. Maintain steaming for five minutes by using intermittent heat.

- Rinse each slide individually in a gentle stream of running water until all free stain is washed away
- Flood the slide with the 25 % H_2SO_4 solution for 2-3 minutes.
- Rinse the slide thoroughly with water. Drain off excess water from the slide.
- Flood the slide with 0. 1% Methylene blue for 30 seconds
- Rinse the slide thoroughly with water. Drain excess water from the slide. Allow smear to air dry. Do not heat or use blotting paper.

EXAMINATION AND REPORTING (ZN MICROSCOPY)

- Use the objective 100x
- Apply one drop of Liquid paraffin oil (heavy) immersion oil to the left edge of the stained smear
- Scan the stained smear systematically from left to right side
- Count AFB in low positive smears for quantification. (Scanty &1+)
- Always search for useful areas, i. e. those containing mucoid threads and pus cells; do this by moving up or down when arriving at an almost empty area, till another useful zone has been found, then continue moving to the left.
- Grade the smear according to WHO guidelines (Table-1)
- Place the slide smear-down on a piece of absorbent paper (absorbent tissue paper,) after examination; let the oil soak in and do not rub
- At the end of the day, store the slides in a slide box
- Do not write the result on the slide
- Clean the objective lens at the end of each day using lens or soft tissue

Reporting: The number of bacilli seen in a smear reflects severity of illness and patient's infectivity.

Table: Grading Chart for ZN Microscopy (100x oil immersion objective and 10x eye piece)

ZN staining grading (RNTCP)	Reporting /Grading

>10 AFB/field after examination of 20 fields :	Positive ,3+
1-10 AFB/field after examination of 50 fields :	Positive, 2+
10-99 AFB/100 field :	Positive, 1+
1-9 AFB/100 field :	Positive, Scanty
No AFB per 100 fields :	Negative

STAINING FOR MALARIA PARASITES: STAINING THICK AND THIN BLOOD SMEARS

PRINCIPLE: Leishman Stain is a neutral stain for blood smears which was devised by the British surgeon W. B. Leishman (1865–1926). It consists of a mixture of eosin (an acidic stain), and Methylene blue (a basic stain) in Methyl alcohol and is usually diluted and buffered during the staining procedure. It stains the different components of blood in a range of shades between red and blue. It is based on a methanolic mixture of "polychromed" Methylene blue and eosin. The methanolic stock solution is stable and also serves the purpose of directly fixing the smear eliminating a prefixing step.

MATERIALS REQUIRED FOR THE LEISHMAN STAIN

- Leishman Stain (Stock Solution)
- Microscopic Glass Slide
- Phosphate buffer (pH 6.8)
- Graduated pipettes
- Measuring cylinder
- Distilled Water
- Pasteur pipette
- Coplin Jar
- Blood Specimen – The specimen used usually consists of fresh whole blood collected by finger puncture (capillary puncture) or the EDTA anticoagulated whole blood, collected by venipuncture and it should be less than 1-hour old for better results.

PREPARATION OF PERIPHERAL BLOOD SMEAR

A Peripheral blood smear (PBS) or Blood film is required to be made from capillary blood or from a drop of blood from an EDTA (anticoagulated) blood sample. Blood smears are needed for microscopic examination of the blood.

Commonly, two types of blood smears are prepared for the diagnosis as well as prognosis of any abnormalities (if present). The Thin blood smear is prepared for studying the morphology of the blood cells and for the identification of microbial agents. The Thick blood smears are prepared for detecting the Blood parasites such as *Plasmodium* spp. (Malaria parasite), *Wuchereria bancrofti & Brugia malayi* (Lymphatic filariasis), *Leishmania donovani* or other species of *Leishmania* (Leishmaniasis), *Babesia* spp. (Babesiosis) etc.

THIN PERIPHERAL BLOOD SMEAR METHOD

PRINCIPLE OF THIN PERIPHERAL BLOOD SMEAR METHOD

The Thin Peripheral Blood smear is made by placing a well-mixed drop of blood 1 to 2 mm in diameter & 1/4 inch from the edge of the clean microscopic glass slide. The drop should be in the center line of the glass slide. These margins are left onto the glass slide to get a region where the cells are spaced far enough apart to be counted and differentiated. Using a second slide as the spreader, the blood is streaked into a thin film in the tongue-like shape and allowed to dry. The blood smear is then fixed and stained with Romanowski stain for microscopic examination.

MATERIALS REQUIRED FOR THIN PERIPHERAL BLOOD SMEAR METHOD

- Syringe & needle
- EDTA vial
- Tourniquet
- 70% isopropyl alcohol
- Sterile Lancet
- Microscopic glass slides

PROCEDURE FOR THIN PERIPHERAL BLOOD SMEAR METHOD

- Collect the blood sample from the capillary or venipuncture
- Now, if the blood sample is obtained by venipuncture, use a capillary tube to transfer a drop of blood from the tube onto the clean grease-free microscopic glass slide. If a finger puncture or heel puncture is made, discard the first drop of blood and then dispense a drop of blood from the puncture site onto the clean microscopic glass slide. Place the drop of blood in the center on one side of the glass slide leaving about 1 cm margins.
- Place the specimen glass slide on a flat surface and hold it with the index finger and the thumb of the left hand (for Right-handed peoples). Now, Place a smooth, clean edge of the spreader slide on the specimen slide at an angle of about 30° – 45°.
- Move the spreader slide toward the drop of blood until the contact is made with the drop of the blood at the specific angle. Then move the spreader slide smoothly and rapidly forward over the specimen slide, drawing the blood behind it into a thin film that should be tongue-shaped.
- Allow the blood smear to air-dry completely. Do not blow air on the slide from any source in an effort to enhance drying, it may distort the smear.
- Using a lead pencil or glass marking pencil, write the Name, Identification no. and the Date on the frosted end of the slide. Do not use a wax pencil or marker or any Pen as it dissolves and washed out during the staining process.

THICK PERIPHERAL BLOOD SMEAR METHOD

PRINCIPLE OF THICK PERIPHERAL BLOOD SMEAR METHOD

A Thick blood film smear requires a large volume of blood as compared to Thin blood films Which enable the more efficient detection of parasites in the blood specimen. A thick blood smear is made by spreading a large blood drop in a small area of about 1 cm which provides a better opportunity to detect various parasitic forms against a more transparent background.

MATERIALS REQUIRED FOR THICK PERIPHERAL BLOOD SMEAR METHOD

- Syringe & needle
- EDTA vial
- Tourniquet
- 70% isopropyl alcohol
- Sterile Lancet
- Microscopic glass slides

PROCEDURE FOR THICK PERIPHERAL BLOOD SMEAR METHOD

- Collect the blood sample from the capillary or venipuncture
- Now, if the blood sample is obtained by venipuncture, use a capillary tube to transfer a Large drop of blood from the tube onto the clean grease-free microscopic glass slide. If a finger puncture or heel puncture is made, discard the first drop of blood and then dispense a drop of blood from the puncture site onto the clean microscopic glass slide. Place the Blood drop in the center of the glass slide.
- Place the specimen glass slide on a flat surface and hold it with the index finger and the thumb of the left hand (for Right-handed peoples).
- Now, spread the blood evenly in a smear or film of about 10 mm wide and about the thickness through which you are able to read the words when the slide is placed on a printed paper.
- Allow the blood smear to air-dry completely. Do not blow air on the slide from any source in an effort to enhance drying, it may distort the smear.
- Using a lead pencil or glass marking pencil, write the Name, Identification no. and the Date on the frosted end of the slide. Do not use a wax pencil or marker or any Pen as it dissolves and washed out during the staining process.
- Now, cover the well dried, thin blood smear with undiluted Leishman Stain solution by counting the drops of Leishman stain.
- Let it stand for 2 minutes, the methanol present in the stain fixes the smear onto the glass slide.

- After 2 minutes, add twice the amount of distilled water or Phosphate buffer solution and mix the content by swirling or by blowing gently. Incubate the slides for at least 10 min at 37 °C. This will stain the blood cells.
- Rinse the slides thoroughly with Phosphate buffer solution up to 2 minutes or until it acquires a purple-pinkish tinge.
- Air dry the slides in a tilted position so that the water easily remove out of the slides.
- Now you can mount the smears with mounting media, e.g. Gurri's neutral mounting media or any other mounting medium which do not decolorizes the smear. Do not use Canada balsam as it may decolorize the smear.
- Let it dry in air for few hours and then observe the slides under oil immersion objective lens of the microscope.

ROUTINE STOOL MICROSCOPY: Stool Faecal specimens for the aetiological diagnosis of acute infectious diarrhoeas should be collected in the early stage of illness and prior to treatment with antimicrobials.

A stool specimen rather than a rectal swab is preferred.

- The faeces specimen should not be contaminated with urine.
- Do not collect the specimen from bed pan.
- Collect the specimen during the early phase of the disease and as far as possible before the administration of antimicrobial agents.
- 1 to 2 gm quantity is sufficient.
- If possible, submit more than one specimen on different days.
- The fresh stool specimen must be received within 1-2 hours of passage. Iodine staining for ova and cysts Iodine staining for ova and cysts
- On a clean glass slide place one drop of normal saline and one drop of 2% iodine solution at two different sites.
- Mix a portion of stool first with normal saline and then with iodine solution with the help of a wire loop or applicator.
- Place coverslips on both the emulsions.
- Examine the preparations under 10x and 40x of the microscope for various ova and cysts.

ALBERT STAINING:

Ingredients and preparations

Albert stain I:

- Toluidine blue 0.15 gm
- Malachite green 0.20 gm
- Glacial acetic acid 1.0 ml
- Alcohol(95%) 2.0 ml
- Distilled water 100 ml

Grind and dissolve the dyes in alcohol, add water and then add acetic acid. Let the mixture stand for 24 hours and then filter.

Albert stain II

- Iodine 2.0 gm
- Potassium iodide 3.0 gm
- Distilled water 300 ml

Dissolve iodine and potassium iodide in water by grinding in a mortar with a pestle. Filter through a filter paper.

Staining procedure

- Cover the heat-fixed smear with Albert stain I. Let it stand for two minutes.
- Wash with water.
- Cover the smear with Albert stain II. Let it stand for two minutes.
- Wash with water, blot dry and examine.

USES: To demonstrate metachromatic granules in *C.diphtheriae*. These granules appear bluish black whereas the body of bacilli appear green or bluish green.

INDIA INK STAINING:

Staining procedure

- Place a loopful of India ink on the side of a clean slide.
- A small portion of the solid culture is suspended in saline on the slide near the ink and then emulsified in the drop of ink, or else, mix a loopful of liquid culture of specimens like CSF with the ink.
- Place a clean cover slip over the preparation avoiding air bubbles.
- Press down, or blot gently with a filter paper strip to get a thin, even film.
- Examine under dry objectives followed by oil immersion.

USE: To demonstrate the capsule which is seen as an unstained halo around the organisms distributed in a black background. This is employed for fungal diagnostics especially for Cryptococcus neoformans.

HEMOCCULT FECAL OCCULT BLOOD TEST

PRINCIPLE: Hemocult is a screening procedure for fecal blood and consists of a cardboard slide with guaiac-impregnated paper in a cardboard frame which permits sample application to one side with development and interpretation on the reverse side. Test is based on the oxidation of phenolic compounds present in the guaiac to quinones resulting in production of the blue color. When a fecal specimen containing occult blood is applied to the test paper, contact is made between hemoglobin and the guaiac. A pseduoperoxidase reaction will occur upon the addition of the developer solution, with a blue chromagen formed proportional to the concentration of hemoglobins. The color reaction will occur after 30 seconds.

MATERIALS:

- Hemoccult Slide(s) CAUTION: Protect from heat, sunlight, humidity, fluorescent light, U.V. radiation, excessive air flow or volatile chemicals; store at 15 - 30°C Do not refrigerate or freeze.
- Wooden applicator sticks
- Hemoccult Fecal Occult Blood Developer <6% Hydrogen Peroxide and Denatured Alcohol
- Stool collection container (for home collection)

SPECIMEN:

- A thin smear of stool is applied to impregnated filter paper using a wooden applicator stick. Specimens are usually collected in groups of three on consecutive days. A clinician may also collect a specimen in a patient examination.

PROCEDURE:

- Hemoccult slides: The patient may collect the specimen and send to the lab, and the technician will apply a thin smear of stool specimen to the filter paper test areas on the Hemoccult slide(s) using wooden applicator sticks.
- If the specimen is thick and/or rehydrating the specimen using 2-3 drops of saline may enhance the sensitivity of the test.
- Open perforated section on back labeled "FOR LABORATORY USE ONLY."
- Apply two drops of developing solution to sample "A" (right side).
- Read results after 30 seconds:
  a) A trace of blue is positive for occult blood.
  b) No indication of blue is negative for occult blood.
- Repeat step 4 for sample "B" (left side).
- For Quality Control, apply one drop of developing solution to each control spot (positive and negative red rectangles at bottom of slide). Read results after 30 seconds and within 2 minutes.

LIMITATIONS:

- It is important that the slides be read 30 seconds after developer has been added and within 2 minutes.
- Results obtained with this test cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology.
- False negative tests may be obtained since most bleeding occurs intermittently.
- Fecal occult blood tests are designed as a preliminary screen and are not intended to replace other diagnostic procedures. This method will detect only hemoglobin released upon hemolysis of the red cell. Should whole blood be applied to the test paper, it is necessary to hemolyze the red cells by the addition of a drop of water before adding the developer.

EXPECTED RESULTS:

- The guaiac paper tests detect occult blood, but they are not diagnostic for disease. False positive results may result from interfering substances or from, diverticulitis, hemorrhoids, or colitis. Positive tests should be followed by a thorough diagnostic workup. This test will detect 10 mg of hemoglobin per gram of fecal material.

TITLE: PREPARATION OF SERUM FROM WHOLE BLOOD

I. Purpose :

To get serum specimens (from whole blood obtained by venupuncture) to be used for a variety of serological investigations such as CRP,ASO , RPR ,rapid HIV testing etc.

II. Principle :

Serum is obtained when whole blood is allowed to clot and then centrifuged .Separation activity is a function of both centrifugal force and timing.

III. Materials and equipment's required :

1. Centrifuge machine
2. Test tubes
3. Tube Holding rack
4. Micropipette
5. Pipe

IV. Procedure :

- Keep the collection tubes with blood on tube –holding rack
- Keep the collected whole blood in the collection tube for 15-20 minutes in order for clotting to take place .
- Plug the test tube .Keep the collection tube in centrifuge .
- Balance the contents of centrifuge (if required with a separate test tube containing plain water)
- Cover centrifuge with its lid .Switch on the electricity supply.
- Increase the speed slowly until speed reaches 3000 rotation per minute (RPM).
- Allow the centrifuge to spin for about 15 minutes .
- Switch off the centrifuge supply .Allow centrifuge to come to rest.
- Remove the test-tube centrifuge.
- Transfer the separated serum with the help of a transfer pipette into a clean test tube .

V. Safety Precautions:

- Always operate the centrifuge with the lid closed .
- Balance contents before turning on.
- Check for vibrations
- Do not open the lid until the rotor has come to a complete stop
- Keep lids on tubes when spinning

Title: CRP test by SMART NEPHELO (Specific Protein Analyser)

Intended use: This reagent is intended for in-vitro quantitative determination of C-reactive protein in human whole blood. Serum or plasma by immunoturbidimetry.

Clinical significance :

CRP-(C-Reactive Protein) is a cytokine –induced .Acute phase protein that increases in concentration as a result of inflammation.CRP levels in the body has been used as a marker or indicator of infections and inflammation .The assay of CRP is more sensitive than the erythrocyte sedimentation rate(ESR) and leukocyte count .The CRP levels rise and return to reference ranges more rapidly after the disease has subsided.

Principle: This is a latex enhanced turbidemetric immune assay .CRP species binds to specific anti-CRP antibodies. Which have been absorbed to latex particles and agglutinates .The agglutination is proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentrations.

A calibration curve is prepared for serum CRP. When test correlated to serum concentration by: CRP (of whole blood)/(1-HCT/100).

Reagent Composition

CRP R1:

Glycine buffer

CRP R2:

Latex suspension coated with anti-CRP antibodies.(rabbit polyclonal antibody).

Storage and stability:

The sealed reagents are stable up to the expiry date stated on label.When stored at 2-8°C .

Linearity:

The reagent is linear up to 150 mg/L. If the concentration is greater than linearity .Dilute the sample with normal saline and repeat assay. Mulitply the result with dilution factor.

Normal Range:

It is recommended that each laboratory should establish its own reference values.The following value may be used as a guide line.Serum up to 8 mg/L.

Preparation and stability of working reagent:

Reagent R1 & Reagent R2 is ready use. Note: once opened the reagents are stable for 45 days.

Precaution:

To avoid contamination. Use clean laboratory wares, avoid direct exposure of reagent to light.

Sample:

Fresh serum: 4ul

Procedure:

1. Add 160 ul R1 into cuvettte (supplied waith reagent)
2. Put the cuvette on incubator more than 5 min or keep it at room temperature more than 15 min.
3. Add pre-processed sample 4ul into cuvette.
4. Insert calibration card into machine and put cuvette into machine measurement well, start measurement.
5. After about 30 sec .add 40 ul R2 into cuvette when incubation is finished .
6. After about 120 sec ,test will finish.

Interferences:

No interfere for

Hemoglobin	up to 500 mg/dL
Conjugated bilirubin	up to 30 mg/dl
Triglycerides	up to 3000mg/dl
Rheumatoid factor	up to 560 IU / Ml

Title: ASO test by SMART NEPHELO (Specific Protein Analyser)

Intended use: This reagent is intended for in vitro quantitative determination of Anti-Streptolysin-O

Clinical significance :

β- haemolytic streptococcus bacteria especially group A,C and G, produce an exotoxin known as Streptolysin-O .People infected with this bacterium produce an antibody known as Anti Streptolysin –O(ASO) .Measuring the levels of ASO is effective for diagnosing ,judging the

progress of medical treatment and assessing the recovery from diseases like rheumatic fever ,acute glomerulonephriits and tonsillitis.

Principle:

When an antigen-antibody reaction occurs between ASO in the sample and streptolsyin –O which has been sensitized to latex particles ,agglutination occurs .The agglutination is proportional to the quantity of ASO in the sample .The actual concentration is determined by interpolation from a calibration curve prepared from calibrators of known concentration.

Reagent composition :

R1: Glycine buffer solution

R2 : ASo latex suspension particles coated with Streptolsyin-O

Storage and stability:

The sealed reagents are stable up to the expiry date stated on the label,when stored at 2-8°C.

Linearity:

The reagent is linear up to 800IU/mL. If the concentration is greater than linearity ,dilute the sample with normal saline and repeat the assay. Mulitply the result with dilution factor.

Normal Range: It is recommended that each laboratory should establish its own reference values.

The following value may be used as guide line. Serum up to 170 IU/mL (adults) and 100 IU/mL (children < 5 years)

Preparation:

Reagent 1 & Reagent 2 are ready to use .

Note: Once opened the reagents are stable for 45 days.

Precaution:

To avoid contamination use clean laboratory wares. Use clean dry disposable pipette tips for dispensing .Close reagent bottles immediately after use .Avoid direct exposure of reagent to light.

Sample:

Serum .Volume: 4ul.

Procedure:

1. Add 160 ul R1 into cuvette (supplied with reagent)
2. Put the cuvette on incubator more than 5 min or keep it at room temperature more than 15 min.
3. Add pre-processed sample 4ul into cuvette.
4. Insert calibration card into machine and put cuvette into machine measurement well , start measurement
5. After about 30 sec ,add 40 ul R2 into cuvette when incubation is finished .

6. After about 120 secs ,test will finish.

Interferences:

No interfere for

Hemoglobin	up to 500 mg/dL
Conjugated bilirubin	up to 30 mg/dl
Unconjugated bilirubin	up to 30mg/dl

Title: RF test by SMART NEPHELO (Specific Protein Analyser)

Intended use:

This reagent is intended for invitro quanititative determination of Rheumatoid factor in Serum.

Clinical significance:

Rheumatoid Factor (RF) is an auto antibody against human IgG commonly seen in sera ,particularly in patients with rheumatoid arthritis .The measurement of RF value is useful in evaluating the diagnosis ,effects of therapy and prognosis of RA ,systemic lupus erythematosus,Chronic hepatopathy.

Principle:

When a sample containing rheumatoid factor is added to denatured human IgG which has been sensitized to latex particles ,antigen-antibody reaction occurs leading to agglutination.The agglutination is proportional to the quantity of RF in the sample .The actual concentration is determined by interpolation from a calibration curve prepared from known value calibrators.

Reagent Composition:

R1:

Glycine buffer Solution

R2 :

Latex suspension coated with denatured human IgG

Storage and stability:

The sealed reagents are stable up to the expiry date stated on the label ,when stored at 2-8°C .

Linearity:

The reagent is linear upto 160IU/mL .If the concentration is greater than linearity ,dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Note: Lower detection limit:< 3IU/Ml

Normal Range:

It is recommended that each laboratory establish its own reference values.The following value may be used as a guideline .Serum up 20IU/mL

Preparation of reagent:

Reagent 1 and Reagent 2 are ready to use.

Note : Once opened the reagents are stable for 45 days.

Precaution:

To avoid contamination, use clean laboratory wares .Avoid direct exposure of reagent to light and heat.

Sample:

Serum volume :4ul per time

Procedure:

1. Add 160 ul R1 into cuvette (supplied with reagent)
2. Put the cuvette on incubator more than 5 min at 37°C.
3. Add pre-processed sample 4ul into cuvette.
4. Insert calibration card into machine and put cuvette into machine measurement well , start measurement
5. After about 30 sec ,add 40 ul R2 into cuvette when incubation is finished .
6. After about 120 secs ,test will finish.

Interferences:

No interfere for

Bilirubin	up to 20mg/dL
Hemoglobin	up to 16 mg/dl
Lipids	up to 1000mg/dl

Title: For detection of Malaria antigen (HRP-II/PlDH) P.v./P.f. by BeneSphera ™ Malaria P.v./P.f. RAPID CARD TEST (LATERAL FLOW)

Intended use:

BeneSphera ™ Malaria P.v./P.f. is a rapid ,self-performing ,qualitative ,two site sandwich immunoassay utilizing whole blood for the detection of *P.falciparum* specific histidine rich protein-2(P.f HRP-2) and *P.Vivax* malaria and *P.falciparum* malaria in areas with high rates of mixed infections.

Principle:

The **BeneSphera ™ Malaria P.v./P.f.** is adopted dual color system ,contains a membrane strip pre-coated with the antibodies specific to histidine –rich protein (HRP-II) of P.falciparum on Pf test line and specific to the lactate dehyrogenase (pLDH) Plasmodium species of P.vivax on Pv test line separately .Another HRPII and /or pLDH specific antibodies are conjugated to colloidal gold,which is dispensed on conjugate pad. The conjugates react with HRP II and pLDH in the sample.

The antigen-conjugate complex forms a visible black band on Pf and/or Pan test line separately with moving along the membrane .On the control line ,anti bovine serum albumin antibody (BSA) is coated ,which capture BSA conjugated gold particle to appear purple/red band. The control line,purple /red , is used for procedural and should always appear if the test procedure os performed correctly.

Kit Contents :

1. Test device Malaria P.v./P.f. card
2. Assay buffer
3. Sample applicator

Storage and stability:

The sealed pouches in the test kit & the kit components may be stored between 2-30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE.

Precautions:

Read the instructions carefully before performing the test. For in vitro diagnostic use only. NOT FOR MEDICINAL USE .Do not use beyond expiry date .Do not inter mix reagents from different lots .Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.

Specimen collection and storage :

Fresh blood from finger prick/vein puncture should be used as a test specimen.However ,fresh anti coagulated whole blood may also be used as a test sample and EDTA or Heparin or may also be used as a test sample and EDTA or Heparin or Oxalate can be used as suitable anti-coagulant.The specimen should be collected in a clean glass or plastic container .If immediate testing is not possible then the specimen may be stored at 2-8°C for up to 72 hours before testing .Clotted or contaminated blood samples should not be used for performing the test.

Test Procedure:

1. Allow all kit components and specimen to room temperature prior to testing.
2. Remove the test device from foil pouch and place it on a flat, prior to testing.
3. Clean the fingertip and prick the finger with lancet .

Or

With a 5ul capillary pipette provided ,draw whole blood specimen and then transfer drawn whole blood into the small sample well.

4. Add 3-4 drops of essay diluents into the square assay diluents well.
5. Wait of 30minutes and read result.
   Caution: Don't read test results after 30 minutes.Reading too late can give false results.

Interpretation of Results:

Negative : The presence of one purple/red band("C" Control line) within the result window indicates a negative result.

Invalid: If the control band fail to appear within the result window,the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated.It is recommended that the specimen be retested.

Positive :

- P.falciparum (Pf) positive: The appearance of two color bands (black "Pf" Test line and purple/red "C" Control line) or three band ("Pf","Pv" Test line and "C" control line) within the result windo indicates a Pf positive result.
- P.vivax (Pv) positive: The appearance of two color bands (black "Pv" and Purple /red "C" control line) within the result window indicates may be mixed infection.
- **Note:** There is no meaning attributed to line color intensity or width.

Title: Widal Test (Typhokit-S) for the diagnosis of Enteric Fever

Staffs able to perform test :

Insupervised : Technicians on basis of rotational shift duty in Central laboratory in CRH.

Under supervision: Students.

Value of the test: Determination and titration of circulating antibodies to Salmonella typhi (O& H antigen) and S.paratyphi (AH & bh antigen) in serum of patient's with suspected enteric fever.

Limitation of the test: The value of the Widal test in diagnosing Enteric Fever in Endemic areas remain controversial. Some expresses the view that the test lacks standardization and adequate sensitivity and specificity to be clinically useful, while others consider the test to have diagnostic value when judged with clinical findings and with a knowledge of normal 'O' and 'H' agglutinin titres in the local population i.e " Baseline titres".

Principle:

It is based on the principle of direct agglutination ,which provides a simple way of qualitative and semi-quantitative estimation of antibodies to *S. Typhi* (O & H) and *S. paratyphi* (AH& BH) . When patient's serum (containing antibodies to *S. Typhi* and *S. paratyphi*) is mixed with the

respective antigens .Visible agglutination indicates the presence of antibodies to the particular antigen (O,H, AH & BH) .A rising titre of antibodies is indicative of enteric fever.

Collection and type of sample:

Type of specimen : Sufficient serum sample obtained from 3-5 ml patient's venous blood collected aseptically using disposable syringe into a clean ,dry ,plain tube /vial without any anticoagulants.

Note:

i. Serum must be free from red cells .
ii. Must not be heated
iii. Whenever possible,collect a second sample 7-10 days later to test for a rise in antibody titre.

Label: Label the specimen properly with patient's name & hospital identification number.

Preferred time of collection of sample:

At the end of first week of fever as agglutinins usually appear by end of first week, and increase steadily till the third or fourth week after which it declines gradually .

Note: (i). Maximum titre is found in third week of fever .

(ii) . Specimen taken earlier than first week may give negative result .

(iii). Specimen taken at the later stage of fever may show fall in titre instead of rise.

Storage of sample: The sample can be stored at 2-8°C for 48 hours incase of delay in testing.

Materials required:

- Sterile test tube
- Normal saline
- Centrifuge
- Micropipette
- Pipette tips
- Disposable tips
- Disposable gloves
- TYPHOKIT-S-Kit contents:
    - S.typhi 'O' antigen
    - S.typhi 'H' antigen
    - S.paratyphi 'AH' antigen
    - S.paratyphi 'BH' antigen
    - Positive control
    - Glass slide
    - Insert

Storage and stability of test kit:

Typhokit-S reagents are stable upto expiry date mentioned on the labels,when stores at 2-8°C .DO NOT FREEZE.

Quality Control:

- Known positive and negative sera can be used for routine performance check of kit.
- Report the test results only when the controls give the expected results.

Method of test:

1. Widal Screening Slide Test (Qualitative) :
    - Take clean and dry Typhokit-S slide.
    - Place one drop (50ul) of undiluted test serum on each of the first four circles.
    - Add one drop each of antigens O,H ,AH and BH in circles 1,2 ,3 and 4 respectively .
    - Add one drop each of antigens O,H AH and BH in circles 1,2,3 respectively .
    - Mix the contents of each circle with separate mixing sticks and spread to fill the whole area.
    - Rock the slide slowly for one minute and observe for agglutination under bright light.

If the agglutination is visible within one minute ,proceed for the estimation of the titre of the appropriate antibody by quantitative method.

2. Semi-quantitative Slide test: (For screening titre of specific antibody)
    - Take clean and dry typhokit- S slide and proceed as follows:

Circle	1	2	3	4	5
Serum volume	80ul	40ul	20ul	10ul	5ul
Appropriate antigen	1 drop	1 drop	1 drop	1 drop	1 drop
Equivalent tube titre	1:20	1:40	1:80	1:160	1:320

    - Mix and rotate for one minute and observe for agglutination under good light.

3. Confirmatory Semi-Quantitative Tube Test:
    - In clean, dry tubes (Dreyer's tube : narrow tube with a conical bottom for 'H' agglutination and Felix tube: short round bottomed tube for 'O' agglutination) dilute each serum sample as follows:

TEST TUBE	1	2	3	4	5	6	7	8
Normal saline	1.9 ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml

Patients serum	0.1 ml	-	-	-	-	-	-	-
Transfer diluted serum	-	1ml	1ml	1ml	1ml	1ml	1ml	-
Appropriate antigen	1 drop	1 drop	1 drop	1drop	1 drop	1drop	1 drop	1 drop
Tube titre	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Saline control

- Mix well and incubate at 37°C for 16-20 hours and observe for agglutination.
- The highest dilution of the serum showing clearly visible agglutination (carpet formation) with naked eye indicates the antibody titre against that particular antigen .
- O antigen shows granular agglutination at the bottom of the tube and H, AH ,& BH antigens shows loose,cotton wool floccular appearance .
- Control tube(8) show a compact deposit (button formation).

  Note:
  - Control tube (8) containing the antigen and normal saline to check for auto-agglutination.
  - Saline control should remain unchanged and is suggestive of negative test results.
  - The paratyphoid 'O' antigens are not employed as they cross react with S.typhi 'O' antigen (TO) due to their sharing of factor 12.

Interpretation/Reporting of results:

Agglutination titre of 1:80 or more is significant.An increase in titre in 4-5 days after the first test is suggestive of active Salmonella infection.

Disposal method:

4. First run the used slide under tap water.
5. Immerse the slide overnight or atleast for 1 hours in a discard jar containing 0.25% Sodium hypochlorite.
6. Wash the slide with detergent and rinse well in running water and keep it for drying.

Precautions:

- Typhokit-S is for in-vitro diagnostic use only.
- Use clean and dry glass slide
- Serum samples should be clear and free from bacterial contamination.

- Do not use plasma sample.
- Allow the reagents to come to the room temperature prior to use and return the reagents to refrigerator immediately after the use.
- Reagents should be shaken well prior to use .
- Observe the agglutination under bright and good light.

SYPHILIS (CARD)

(IMMUNO CHROMATOGRAPHY METHOD)

INTENDED USE:

This Syphilis Test is intended for "In vitro" qualitative determination of Treponemal antibodies (IgA, IgM, IgG) produced against Treponema pallidum antigen (17KDa,15KDa,47KDa) in human serum/plasma.

PRINCIPLE:

Syphilis Rapid Test is a qualitative membrane device based immunoassay for the detection of TP antibodies (IgA, IgM, IgG) in a serum or plasma. In this procedure, recombinant syphilis antigen (17KDa,15KDa, 47KDa) is immobilized in the test line region of the device. After the specimen is added to the specimen well of the device, it reacts with syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized Syphilis antigen. The double antigen test format can detect IgA, IgG and IgM in specimens. If the specimen contains TP antibodies a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a colored line will not appear in the test region, indicating a negative result. To serve as a procedural control a colored, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

SPECIMEN COLLECTION & PREPERATION:

Use fresh serum, collect blood into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use fresh serum for testing.

TEST PROCEDURE:

1. Remove the test device from its protective pouch (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identification and use the device as soon as possible.

2. Using the dropper provided put 2-3 drops of serum sample into the sample well. Avoid overflowing.
3. Wait for 5-20 minutes and read result. It is important that the background is clear before the result is read.

IMPORTANT NOTE: Do not read results after 30min's PRECAUTION:

1) For in vitro diagnostic use only.
2) Do not use test kit beyond expiry date.
3) The test device should not be reused.
4) Keep out of reach of the children.
5) Do not freeze the kits.
6) Specimen with extremely high concentration of red blood cells, fibrin should be recentrifuged before use.

STORAGE AND STABILITY:

The test kit can be stored at temperature 4 to 30°C in the sealed pouch to the date of expiration. The test kit should be kept away from direct sunlight, moisture and heat.

INTERPRETATION OF RESULTS:

Negative: Only one colored band appears on the control (C) region. No apparent band on the test (T) region.

Positive: In addition to a pink colored control (C) band, a distinct pink colored band will also appear in the test (T) region.

Invalid: A total absence of color in both regions or no colored line on the control(C) region is an indication of procedure error and/or the test reagent deterioration. Repeat the test with a new kit.

LIMITATION:

1. As with all diagnostic tests, all result must be considered with other clinical information available to the physician. A definite clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. This test kit is for the detection of TP antibodies in serum specimen. This test is for in vitro diagnostic use only. Neither the quantitative value nor the rate of increase TP antibodies can be determined by this qualitative test.
3. This kit will only indicate the presence of TP antibodies in the specimen and should not be used as the sole criteria for the diagnosis of TP infection.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative results does not at any time preclude the possibility of TP infection.

5. Positive results should be confirmed be other confirmatory tests.

ENTEROSCREEN-WB

Rapid test for detection of IgM and IgG antibodies to s. typhi in serum/plasma /whole blood

INTENDED USE

ENTEROSCREEN-WB is a rapid, self performing, qualitative, sandwich immunoassay for the detection and differentiation of IgM and IgG antibodies to S. typhi in human serum/plasma or whole blood specimen.

PRINCIPLE

ENTEROSCREEN-WB utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. ENTEROSCREEN-WB is a dual test device assembly comprising of an IgM detection test assembly and an IgG detection test assembly. The conjugate pad of the IgM test assembly consists of two components, Agglutinating sera for human IgM conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. Similarly the IgG test assembly consists of Agglutinating sera for human IgG conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the respective membrane test assemblies, the Agglutinating sera human IgM or the Agglutinating sera for human IgG –colloidal gold conjugated complexes with S. typhi specific IgM or IgG antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit globulin-colloidal gold conjugated. This complex moves further to the test region of the respective test assembly where the specimen is immobilized by the S. typhi specific antigen coated at the test regions of the IgM/IgG device assembly leading to formation of a pink to pink-purple colored band at the test regions of the respective test devices which indicates a positive IgM or IgG test result. The absence of this colored band in either of the test regions indicates a negative test results.

In both the test membrane assemblies the unreacted conjugate and unbound complex, if any move further on the membranes and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membranes at the control region (c), forming a pink to pink-purple colored band. The control band acts as a procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

ENTEROSCREEN-WB kit contains:

A. Individual pouches, each containing-
    1. Dual test device:
    IgM Test Assembly: Membrane assembly pre-dispened with Agglutinating sera for Human IgM – colloidal gold conjugate, rabbit globulin _ colloidal gold conjugate, S, typhi specific antigen and Agglutinating sera for rabbit globulin coated at the Test region ‘T’ and Control region ‘C’ respectively and
    IgG Test Assembly: Membrane assembly pre-dispensed with Agglutinating sera for Human IgG – colloidal gold conjugate, rabbit globulin – colloidal gold conjugate, S.

typhi specific antigen and Agglutinating sera for rabbit globulin coated at the Test region ‘T’ and Control region ‘C’ respectively.
2. Desiccant pouch.
3. Disposable plastic sample applicator.

B. Sample running buffer in a dropper bottle.
C. Package Insert.

STORAGE AND STABILITY

The sealed pouches in the test kit &the kit components may be stored between 4°C to 30°C till duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30°C for the remaining duration of its shelf life.

SPECIMEN COLLECTION AND PREPARATION

1. ENTEROSCREEN-WB uses human serum/plasma/whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. For whole blood, collect blood with suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Whole blood should be used immediately and should not be frozen.
5. Though fresh specimen is preferable, in case of delay on testing, it may be stored at 2°C to 8°C for maximum up to 24hrs.
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Repeated freezing and thawing of the specimen should be avoided.
8. Do not use turbid, lipaemic and hemolysed serum/plasma.
9. Do not use hemolysed, clotted, contaminated, viscous/turbid specimens.
10. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

TESTING PROCEDURE

1. Bring the kit components of ENTEROSCREEN-WB device to room temperature before testing.
2. Open a foil pouch by tearing along the “notch”.
3. Remove the testing device and the sample applicator. Once opened, the device must be used immediately.
4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Carefully dispensed 5µl of either whole blood/serum/plasma into the specimen port ‘A1’ and 5µl of same specimen into the specimen port ‘A2’ of the test device using a

micropipette OR using the 5µl sample applicator provided, dip the sample applicator in the sample container and blot the sample in the sample port 'A1' and then using the same applicator, dip onto the sample container again and blot the sample in the sample port 'A2'.
7. Add five drops each of sample running buffer into buffer port 'B1'&'B2'.
8. At the end of 15 minutes, read the results.

INTERPRETATAION OF RESULTS

Negative Result:

If only one colored band appears in the control region 'C' of both the test windows. It indicates absence of antibodies to S. typhi in the specimen or that the amount of antibodies is below detection limit of the test.

Positive Result:

IgM Positive: In addition to a colored band appearing in the control region 'C' in both the IgM and IgG Test windows, a colored band appears in the Test region 'T' of the IgM test window. The presence of only IgM antibodies is indicative of current acute typhoid infection. The intensity of the test band may be more or less than the control band, depending upon the concentration of antibodies in specimen.

IgM and IgG Positive: In addition to a colored band appearing in the Control region 'C' in both the IgM and IgG Test windows, a colored band observed in Test region of both IgM and IgG Test windows. The presence of both IgM and IgG antibodies indicates acute typhoid fever (in the middle stage of Salmonella typhi infection). The intensity of the test band may be more or less than the Control band, depending upon the concentration of IgM and IgG antibodies in specimen.

IgG Positive: In addition to a colored band appearing in the Control region 'C' in both the IgM and IgG Test windows, a colored band appears in the Test region 'T' of the IgG test window. The presence of only IgG antibodies is indicative of previous Salmonella typhi infection (in which case current fever may not be due to typhoid) or relapse or re-infection. The intensity of the test band may be more or less than the Control band, depending upon the concentration of antibodies in specimen.

Invalid Result: The test is invalid if the control band in both or either one of the devices is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

LIMITATION OF THE TEST

1. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pocket or patches may appear, which do not interfere with the test results. Presence of a band at the test region even if low in intensity or formation is a positive result.
2. The deliberate slow reaction kinetics of ENTEROSCREE-WB is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.

3. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only at 30 minutes. Do not read results after 30 minutes.
4. As with all diagnostic tests, a definite clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
5. ENTEROSCREEN-WB should be used as a screening test in clinically suspected case only, and its results should be confirmed by other supplemental method before taking clinical decisions.
6. In some studies it has been reported that low titre IgM antibodies to S. typhi may persist for about 4 months post infection. Therefore, in endemic area, samples positive yet with low signal intensity should be interpreted with caution, preferably in light of patient history.
7. The following chart would explain the IgM seroresponse in S. typhi infected subjects after onset of fever.

Detectable IgM Response	
Onset of fever	**Percentage positive**
4-6 days	43.50%
6-9 days	92.90%
>9 days	100%

8. A negative result, i.e. the absence of detectable IgM antibody does not rule out or current infection, as the positivity is influenced by the time elapsed from the onset of fever and immunocompetence of the patient. However, if S. typhi infected is still suspected, obtain a second specimen 5-7 days later and repeat the test.
9. High titre Rheumatiod factor may result in a false positive reaction.
10. A low extent of cross reactivity may be observed with S. paratyphi infection.

PROGEN

PROTEUS ANTIGEN SUSPENSION FOR WELL-FELIX TEST (OX19,OX2,OXK).

PURPOSE

PROGEN antigen suspensions employed in the Weil-Felix test, are used for the diagnosis of Rickettsial infection and differential diagnosis in patients with Febrile fever.

PRINCIPLE

The smooth, killed stained PROGEN antigen suspensions are mixed with the patients serum. Antibodies produced due to rickettsial infection if present in the patient serum will react with the stained PROGEN antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of Rickettsial antibodies.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of patients is required prior to sample collection by approved techniques. Do not use haemolysed and turbid serum samples.
2. Blood collected be venipuncture should be allowed to clot naturally. Care should be taken to ensure that the blood sample are fully clotted.
3. Clean and dry glassware, free from detergents must be used for sample collection.
4. Do not heat inactivate the serum.
5. Though freshly collected serum is preferred, samples can be stored at 2-8°C for 24 hours, or frozen for 8 days should a delay in testing occur.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
2. The shelf life of the reagents as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

MATERAIL PROVIDED WITH THE KIT

REAGENT PACK

PROGEN™ OXK Antigen Suspension (REF.:105810005), PROGEN ™ OX19 Antigen Suspension (REF.:105820005), PROGEN ™ OX2 Antigen Suspension (REF.:105830005).

TEST PROCEDURE

1. Bring reagents and sample to room temperature before testing.
2. Shake and mix the PROGEN ™ antigen suspension well before dispensing.
3. The test procedure for PROGEN ™ OXK/ PROGEN ™ OX19/ PROGEN ™ OX2 are identical.

A. Rapid Slide Screening Test
   1. Place a drop of positive control onto a reaction circle of the glass slide.
   2. Place a drop of Physiological saline onto the next reaction circle of the glass slide.
   3. Place a drop of patient sample to be tested onto the next reaction circle (for PROGEN ™ OXK refer note below).
   4. Add one drop of appropriate PROGEN ™ antigen suspension to the reaction circles containing positive control and physiological saline.
   5. Add one drop of appropriate PROGEN ™ antigen suspension to the reaction circles containing the patient serum.
   6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
   7. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

NOTE: The level of agglutinins in normal human serum can be 1:80 or more specially with PROGEN ™ OXK suspension which may give titres up to 1:160. Therefore, it is recommended that in endemic areas for PROGEN ™ OXK reagent instead of 50µl patient serum 5µl which corresponds to 1:320 titre be used in the rapid slide screening test.

B. Semi-Quantitative Slide Method

1. Using a micropipette place 80 µl,40 µl,20 µl,10 µl and 5 µl of patient serum to be tested on 5 different reaction circles on the glass slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, 1:320respectively.
2. Follow step No5-7 of rapid slide screening test. This method is recommended for obtaining quick approximate titres only.

INTERPRETATION OF RESULTS

Rapid Slide Screening Test

Agglutination obtained within one minute is a positive reaction and indicates the presence of the corresponding antibody in the patient serum.

No agglutination is a negative test result and indicates the absence of the corresponding antibody in the patient serum.

Slide Semi-Quantitative Method

The rection obtained are roughly equivalent to those which would occur in a tube agglutination test with serum dilution of 1:20, 1:40, 1:80, 1:160, 1:320 respectively. If a positive reaction is observed it is advisable to confirm the result and establish the titre by a tube test. A tube test is indicated when results do not conform to clinical findings. False results may be obtained if the reagents are not allowed to reach room temperature (22-30°C) before use. False positive reactions are likely if the test is read beyond one minute after mixing.

ANALYSIS OF RESULTS

Agglutination patterns for several rickettsial diseases are shown below:

Infection	Vector	PROGEN ™ antigen suspension		
		OX19	OX2	OXK
Epidemic typhus	Louse	+++	+	-
Murine typhus	Flea	+++	+	-
Endemic typhus	Flea	+++	+	-
Rocky Mountain Spotted fever	Tick	+++	+	-
Tsutsugamushi Fever	Mite	-	-	+++
Scrub typhus Mite	Mite	-	-	+++
Boutonneuse fever	Tick	+	+	+

South African tick-bit fever	Tick	+	+	+
Brills, disease Louse	Louse	Usually neg.	Usually neg.	-/±
Trench fever louse	Louse	-	-	-
Q Fever	Tick	-	-	-

LIMITATIONS

1. The level of agglutination in "normal" human sera can be 1:80 or more, especially with PROGEN TM OXK antigen suspension which may give "normal" titres up to 1:160.
2. Positive reactions due to previous vaccinations, anamnestic response, antibiotic therapy, narcotic addiction, other diseases such as malaria, infectious mononucleosis, typhoid, brucellosis, liver disease and autoagglutinations as well as urinary infection by Proteus, may affect the test results and therefore the result must be judged in the context of the clinical findings.
3. It is recommended to test the suspension as described with known positive and negative control serum with each run of test samples.
4. False results may be obtained if the reagents are not allowed to each room temperature (22-30°C) before use. False positive reactions are also likely if the test is read beyond one minute after mixing.
5. A great number of false positive reactions have been reported in healthy individual with Proteus antigen especially in slide agglutination tests. A titre of less than 1:160 should not be considered significant.

REMARKS

1. Positive result obtained in the slide test should be confirmed with the tube test to establish whether the titre are diagnostically significant or not.
2. Patients occasionally fail to develop any antibodies.
3. Weil-Felix reaction may vary widely from case to case of spotted fever and therefore may be of little help in either detecting the disease or differentiating if from murine typhus.
4. The test is not a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organism.
5. The level of agglutinins in "normal" human sera can be 1:80 or more, especially with PROGEN TM OXK antigen suspension which may give "normal" titres up to 1:160. A rising or falling titres more significant than a single elevated titre.
6. Agglutinins tend to fall rapidly within few months of recovery from an infection and therefore a high titre is useful indication of recent infection.
7. Many serotypes pathogens have common somatic antigens. Agglutination with any of PROGEN TM antigen suspension by the patient serum cannot therefore be taken as a proof of infection by that particular organism but possibility of infection by an organism of similar antigenic constitution should be considered when reporting results.
8. Positive reactions due to previous vaccinations, anamnestic response, antibiotic therapy, narcotic addiction, other diseases such as malaria, infectious mononucleosis, typhoid, brucellosis, liver disease and autoagglutinations as well as urinary infection by Proteus, may affect the test results and therefore the result must be judged in the context of the clinical findings.

9. It is recommended to test the suspension as described with known positive and negative control serum with each run of test samples.
10. False positive results are likely if the test is read more than one minute after mixing on the slide test.
11. Any deviation in test procedure could lead to variable results.
12. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
13. Use a separate disposable tip for each sample to prevent cross contamination.
14. Turbid and contaminated sera should not be used for testing.
15. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.
16. Reagent vials that have leakage/ breakage problem should be discarded.
17. Only qualified and well trained staff should use the reagents.
18. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
19. The performance of the antigen suspension should be validated occasionally using positive control. Good physiological saline may be used as a negative control.

RAPID IMMUNOCHROMATOGRAPHY TEST FOR HIV – 1 AND HIV – 2

PROPOSE

QUAL PRO HIV, is a rapid, 3rd generation, qualitative, sandwich immunoassay for simultaneous and differential detection of total antibodies i.e. IgG, IgM, IgA etc. to HIV-1 and HIV-2 virus in human serum/ plasma. For professional use.

PRINICIPLE

QUAL PRO HIV utilizes the principle of immunochromatography, a unique two- side immunoassay on a nitrocellulose membrane. Highly purified antigens – gp41, gp120 and p24-0 fusion polypeptide, representing HIV-1 and HIV -2 group "0" and synthetic peptide gp36 representing HIV-2 are stripped on the membrane as two separate test bands. An assay control for the third band. Similar antigens are also coated on colloidal gold. A unique combination of synthetic peptides and recombinant antigens reduces cross -reactivity and enable better

discrimination between HIV-1 and HIV-2 samples. As the test specimen floes through the membrane test assembly, the highly specific HIV-1/2 antigens- colloidal gold conjugate complexes with the HIV1/2 specific antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit IgG-colloidal gold conjugate. This complex moves further on the membrane to the test region where it is immobilized by the HIN-1/2 antigen coated on the membrane at two separate test regions for HIV-1 and HIV-2. This leads to the formation of colored band (s). The unreacted conjugate and unbound complex, if any, along with rabbit IgG gold conjugate move further on the membrane and are subsequently immobilized by the goat anti-rabbit IgG antibodies coated on the membrane at the control region (C), forming a colored band. This control band acts as a procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

QUALPRO HIV kit has the following components.

A. Individual pouched devices each comprising of:
    1. DEVICE. Membrane test assembly: stripped with HIV-1 and HIV-2 specific antigens and goat anti- rabbit IgG along with HIV specific antigen and rabbit IgG gold conjugate.
    2. PIPETTE. Disposable plastic sample applicator.
    3. Desiccant pouch.
B. BUFFER. Sample running Buffer: buffer containing surfactant and preservatives.
C. Package insert.

STORAGE AND STABILITY

QUALPRO HIV is stable up to the expiry date mentioned on the lable when stored at 4 - 30°C. Once the pouch is opened, the membrane test assembly must be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Disinfectant
2. Disposable gloves
3. Biohazard waste container

SAMPLE COLLECTION

1. **QUALPRO HIV** uses human serum/plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.

3. Preferably use fresh sample. However, specimen may be stored refrigerated (2-8°C) for short duration. For long storages, freeze at-20°C or below.
4. If serum is to be used as specimen, allow blood to colt completely. Centrifuge to obtain clear serum.
5. Repeated freezing and thawing of the specimen should be avoided.
6. Do not heat inactivate before use.
7. Do not use turbid, lipaemic and hemolyzed serum/ plasma.
8. Do not use hemolyzed, clotted or contaminated specimen.
9. Specimen containing precipitates or particulate matter must be centrifuge and the supernatant only used for testing.
10. Refrigerated specimen must be brought to room temperature prior to testing.

PRECAUTIONS

1. For in vitro diagnostic uses only. NOT FOR MEDICINAL USE.
2. Bring all reagents and specimen to room temperature before use.
3. Do not use beyond expiry date.
4. Read the instruction carefully before performing the test.
5. Handle all specimen as if potentially infection.
6. Do not pipette any material by mouth.
7. Do not eat, drink or smoke in the area where testing is done.
8. Use protective clothing and wear gloves when handling samples.
9. Use absorbent sheet to cover the working area.
10. Immediately clean up any spills with sodium hypochlorite.
11. Dispose off all the reagents and material used as if they contain infectious agent.
12. Do not mix components of one lot with another.
13. If desiccant colour at the point of opening the pouch has turned from blue to white, another test assembly must be run.

TEST PROCEDURE

1. Bring the sealed aluminum foil pouch of **QUALPRO HIV** membrane test assembly to room temperature.
2. Open a foil pouch by tearing along the "notch".
3. Remove the membrane test assembly and the sample applicator. Once opened, the membrane test assembly must be used immediately.
4. Label the membrane test assembly with specimen identity.
5. Place the membrane test assembly on a flat horizontal surface.
6. Carefully dispense one drop (25µl) of serum/ plasma into the specimen well 'S' using the sample applicator provided.
7. Add three drops of sample running buffer into the same well 'S'.
8. Observe the development of visible coloured band at test regions (1 for HIV-1 and/or '2' for HIV-2).
9. Positives result may be observed within 20 minutes.

10. The test should be considered invalid if the control band ‘C’ dose not appear. The test is also invalid if neither the control nor the test bands appears. Repeat the test with a new **QUALPRO HIV** membrane teat assembly.

INTERORETATION OF RESULT

NEGATIVE: A colored band appears only in the control area marked ‘C’.

HIV-1 POSITIVE: A colored band appears in the control area as well as in the area marked ‘1’. The sample is reactive for HIV-1.

HIV-2 POSITIVE: A colored band appears in the control area as well as in the area marked ‘2’. The sample is reactive for HIV-2.

HIV-1 & HIV-2 DUAL POSITIVE: A colored band appears in the control area as well as in the area marked ‘1’ &’2’. This indicate a mixed infection.

INVALID: The test should be considered invalid if the control band ‘C’ does not appear. The test is also invalid if only the test band and no control band appear. Repeat the test with a new **QUALPRO HIV** membrane test assembly.

RAPID IMMUNOCHROMATOGRAPHY TEST FOR HCV HUMAN SERUM/PLASMA

PURPOSE

Flaviscreen plus is a a rapid, third generation two- side sandwich immunoassay for the detection of total antibodies specific to hepatitis C virus (HCV in human serum/plasma). The test employs a genotype cross- reactive multi-epitope recombinant antigen derived from the Core, NS3, NS4 and NS5 region of multiple HCV genotypes. The double antigen sandwich system ensures detection of all anti-HCV antibody isotopes (viz. IgG, lgM,igA etc)to all major HCV genotypes.

PRINCIPLE

Flaviscreen plus utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immunochromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components a multi-epitope HCV recombinant antigen conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test

specimen flows through the membrane test assembly that membrane test assembly the HCV recombinant antigen-colloidal gold conjugate complexes with the anti-HCV antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit globulin – colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another multi- epitope HCV recombinant antigen coated on the membrane leading to formation of a pink to pink-purple coloured band. The absence of this coloured band in the test region indicates a negative test result.

The unreacted conjugated and unbound complex, if any, along with rabbit globulin-gold conjugate moves further on the membrane and are subsequently immobilized by the agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. This control band acts as aa procedural control and serves to validate the results.

REAGENTS AND MATERIALS SUPPLIED

Flaviscreen plus kit has following components:

a. Individual pouches each containing:
    1. Devices: membrane test assembly: Stripped with multi-epitope HCV recombinant antigen and agglutinating sera for rabbit globulin along with HCV specific antigen and rabbit globulin-gold conjugate. Each membrane test assembly is individually pounced.
    2. Desiccant pouch.
b. **BUFFER**: Sample running buffer in a dropper bottle.

STORAGE AND STABILITY

Flaviscreen plus is stable to the expiry date mentioned on the label when stored at 4-30°C. Once the pouch is opened, the device must be used immediately.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disinfectant
2. Disposable gloves
3. Biohazard waste container
4. Micropipette

SAMPLE COLLECTION

1. **Flaviscreen plus** used human serum/plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. Preferably use fresh sample. However, specimen may be stored refrigerated (2-8°C)for short duration. For long storage, freeze at 20°C or below.
4. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.

5. Repeated freezing and thawing of the specimen should be avoided.
6. Do not heat inactivate before use.
7. Do not use turbid, lipaemic and hemolyzed serum/plasma.
8. Do not use hemolyzed, clotted or contaminated specimen.
9. Specimen containing precipitates or particulate matter must be centrifuges and the clear supernatant only used for testing.
10. Refrigerated specimen must be brought to room temperature prior to testing.

PRECAUTIONS

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE
2. Bring all reagents and specimen to room temperature before use.
3. Do not use beyond expiry date
4. Read the instruction carefully before performing the test.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimen as if potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink or smoke in the area where testing is done.
9. Use protecting clothing and wear gloves when handling samples.
10. Use absorbent sheet to cover the working area.
11. Immediately clean up any spills with sodium hypochlorite.
12. Dispose of all the reagents and material used as if they contain infectious agent.
13. Do not mix components of one lot with another.
14. If desiccant color at the point of opening the pouch has turned from blue to white, another test assembly must be run.

TEST PROCEDURE

1. Bring the sealed aluminum foil pouch of **Flaviscreen plus** membrane test assembly to room temperature.
2. Open a foil pouch by tearing along the “notch”.
3. Remove the membrane test assembly. Once opened, the membrane test assembly must be used immediately.
4. Label the membrane test assembly with specimen identity.
5. Place the membrane test assembly on a flat horizontal surface.
6. Carefully dispense 10µl of serum /plasma into the specimen port “A”.
7. Add three drops of sample running buffer into port “B”.
8. Observe the development of visible colored band at test region.
9. Positive result may be observed within 15-20 minutes. Do not read result after 20 minutes.
10. The test should be considered invalid if the control band “C” does not appear. The test is also invalid if neither the control mor the test bands appear. Repeat the test with a new **Flaviscreen Plus** membrane test assembly.

NEGATIVE

If antibodies to HCV are not present, only one colored band at control window (C) would appear.

POSITIVE

If antibodies to HCV are present, two colored bands appear at test and control (C) windows.

INVALID

The test should be considered invalid if neither the test nor the control bands appear. Repeat the test with a new device.

RAPID IMMUNOCHROMATOGRAPHY TEST FOR HBV HUMAN SERUM/PLASMA: HBsAg

VIRUCHECK one step test for HBsAg is a rapid, qualitative, two site sandwich immunoassay for the detection of hepatitis B surface antigen, a marker for hepatitis b infections, in serum/plasma specimen.

PRINCIPLE

VIRUCHECK one step test for HBsAg utilizes the principle of agglutination of antibodies/antisera with respective antigen in immune-chromatography format along with use of nano gold particles as agglutination revealing agent.as the test sample flows through the membrane assembly within the device, the coloured agglutinating sera for HBsAg-colloidal gold conjugate complexes with the HBsAg in the sample. This complex further on the membrane leading to the test region where it is immobilized by the agglutinating sear for HBsAg coated on the membrane leading to formation of a coloured band which confirm a positive test result. Absence of this coated in the test region indicate a negative test result. The unreacted conjugate and unbound complex if any move further on the membrane and are subsequently immobilized by the agglutinating sera for rabbit globulin coated om the membrane at the region forming a coloured band. The control band serves to validate the test results. The control band formation is based on the 'Rabbit globulin / Agglutinating sera for Rabbit globulin system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED

Each individual pouch contains:

1. Device: contain membrane assembly predispensed with agglutinating sera for HBsAg-colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, agglutinating sera for rabbit globulin at the respective region.
2. PIPETTE : disposable plastic sample applicator.
3. Desiccant pouch.

STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 4°C to 30°C till the duration of the duration of the shelf as indicated on the pouch. DO NOT FREEZE

NOTE

1. For in vitro diagnostic use only. NOT FOE MEDICINAL USE
2. Do not use beyond expiry date.
3. Read the instruction carefully before performing the test.
4. Handle all specimen as potentially infection.
5. Follow standard biosafety guideline and disposal of potentially infective material.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum, inhalation /swallowing may cause harm.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is necessary prior to specimen to specimen collection by approved techniques. Through fresh serum/ plasma is preferable, serum/plasma specimen may be stored 2°to 8°C for up to 24 hours, in case of delay in testing. Do not use haemolysed, turbid or contaminated samples. Turbid samples should be centrifuged and clear supernatant must be used for testing.

TESTING PROCEDURE AND INTERPERTATION OF RESULTS

1. Bring the sealed pouches to room temperature. Open the pouch and remove the device, applicator and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the device and use another device. Once opened, the device must be used immediately.
2. Dispense two drops (50µl) of serum/plasma specimen into the sample well 'S' using the applicator provided. Refrigerated specimen must be brought to room temperature prior to testing.
3. At the end of fifteen minutes read the results as follows:

NEGATIVE

A coloured band appears at the control region 'C'.

POSITIVE

In addition to the control band, a coloured band also appears at the test region 'T'.

INVALID

The teat should be considered invalid if no colored band appears on the device. The test should also be considered invalid if a colored band appears in only at the region 'T' and not at the control region 'C' in such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

6. Specific Information on Haematology & Clinical Pathology:

6.1. HEMOGLOBIN

- Hemoglobin is the main content of the red cell, which is a conjugated protein that gives red colour to the blood.
- 2 ml of EDTA anticoagulated blood is required. Estimation is done by Cyanmethhemoglobin method using automated cell counter.
- Significance of hemoglobin estimation.
- Decrease in hemoglobin below the normal range is an indication of anemia.
- Increase in hemoglobin concentration is seen in diarrhea, vomiting, burns, hypoxic states, polycythemia Vera etc.
- Used to diagnose, type and user degree of amenity.
- Report will be made available within 2hr of blood collection.
- The primary sample is kept for 24 hrs and therefore retesting after 1 day is not possible.
- Remaining blood is discarded by incineration.

6.2. PACKED CELL VOLUME (PCV) OR HEMATOCRIT

- Hematocrit is the ratio of volume of erythrocytes to that of the whole blood.
- It is expressed as a percentage
- There are two methods of measurement of PCV.

1. Macro method using Wintrobe‟s tube

2. Micro method using capillary tubes

3. Automated method.

Tube is centrifuged and the reading is obtained.

- 2 ml of EDTA anticoagulated blood is used.
- Readings are obtained after 30 minutes.
- Used for the diagnosis of anaemia and polycythemia.
- Value available in one and half hours.
- Sample is discarded by incineration.

6.3. ERYTHROCYTE INDICES

- Based on the results of tests which measure hemoglobin, packed cell volume and total red cell count, several calculations have been described which given quantitative information about the red cells.
- The purpose of calculating these values is mainly for the classification of anemias.
- The various indices are: -

1. Mean corpuscular volume or MCV
2. Mean corpuscular hemoglobin or MCH
3. Mean corpuscular hemoglobin concentration or MCHC

- Method of estimation is by automated method or manually by using calculations.
- EDTA anticoagulated blood around 2 ml is used.

Indices are used to classify anemias morphologically.

- Report will be made available within one and half hours.
- Sample is kept for 1 day and discarded by incineration.

6.4. ERYTHROCYTE SEDIMENTATION RATE (ESR)

- Venous blood is placed in a vertical tube.
- The red cells sediment or fall to the bottom, this length of fall of column of RBC"s in a given time is called erythrocyte sedimentation rate (ESR).
- 2ml of citrated blood is collected in a vacutainers.
- Methods of ESR estimation are

1. Westergren method

2. Micro ESR method

3. Wintrobe"s method

- An increase in ESR is usually an indication of disease.
- Elevated in cases of chronic inflammatory conditions like tuberculosis, rheumatoid arthritis or malignancy. Can be used to follow a disease activity or response to treatment once a diagnostic is made.
- Report is made available after 1 hour.
- Sample is discarded after 1 day by incineration

6.5. DIFFERENTIAL COUNT

- Is a method where by the number of different types of white blood cells present in a blood sample are counted.

- WBC"s are expressed as total number of cells counted.

- 2 ml of EDTA anticoagulated blood is collected.

- Method of differential count

1. Automated cell count

2. Manual method / smears.

- Increase in neutrophil count is called neutrophilia and seen in acute pyogenic infections, acute hemolysis or hemorrhage, strenuous exercise, stress, pain, chronic myeloproliferative disorders etc.

- Decrease in neutrophil count is called neutropenia and is seen in cases of viral infections, acute leukemias, megaloblastic anemia etc.

- Report will be available within one and half hours.
- Sample will be discarded after 1 day by incineration and retesting after 1 day is not possible.
- Lymphocyte increases in cases of viral infection, children, typhoid, tuberculosis, chronic lymphocytic leukemia etc.,
- Eosinophil count increases in cases of parasitic infections, allergies, eczema, psoriasis etc.
- Total count (TC) may be increased in all the above conditions.
- Total count may be decreased in bacterial, viral or protozoal infections.
- 2 ml of EDTA anticoagulated blood is used and report is available in one and half hours. .

6.6. PLATELET COUNT

- Done using 2ml of EDTA anticoagulated blood.
- Platelets are blood cells, which function in hemostasis to maintain integrity of the blood vessels.
- Methods of obtaining platelet count are

1. Manual method / smear

2. Automated cell count.

- Platelets are increased in physiological states such as exercise and pathological states, such as CML, myeloproliferative disorders, essential thrombocytosis, Post hemorrhagic, postsurgical states, infections, and inflammation.
- Platelets are reduced in aplastic anemia, myeloblastic anemia, acute leukemias, infections etc.
- Report is made available in one and half hours.
- Sample discarded by incineration after 1 day.

6.7. RED BLOOD CELL COUNT

- Erythrocyte count is done, usually by automated cell counter.
- Values are higher in the morning and lowest in the evening.
- 2 ml of EDTA anticoagulated blood is used.

It is helpful to calculate the values to obtain the indices.

- The highest value is seen at birth and increased counts are also seen dehydration, severe burns, chronic lung diseases like emphysema, polycythemia etc.,
- Decreased count is seen in pregnancy, anemia etc.,
- Report is available in one and half hours.
- Sample discarded by incineration after 1 day and retesting after 1 day is not possible.
- Complete blood count includes HB, TC, and DC, ESR (Turn over time – one and half hour).

6.8. PERIPHERAL SEMAR

- 2 ml of EDTA anticoagulated blood is used.
- Blood smears are prepared on a glass slide.
- Used to assess the morphology of the blood cells and to look for abnormal cells.
- Helps in diagnosis and typing of anemia along with other parameters.
- Malaria Parasite can be visualized by this method.
- Helps to confirm thrombocytopenia and decrease or increase in other cell counts.
- Turnaround time – 24 hours.
- Sample discarded after 1 day and retesting is not possible after 1 day.
- Complete hemogram, includes – Hb, TC, DC, ESR, RBC and reticulocyte count and blood indices.

6.9. RETICULOCYTE COUNT

Immature anuclear erythrocytes are called reticulocytes.

- 2 ml of fresh EDTA anticoagulated blood is preferred.
- Reticulocyte count can be estimated by supravital staining and microscopic examination.
- Peripheral blood reticulocyte count indicates effective bone marrow activity.
- Most useful in monitoring anemia and response to therapy.
- The results are expressed as percentage of reticulocytes in relation to total RBC count.
- Turnaround time – 4 hours.
- Sample is described after 2 days and retesting after 1 day is not possible.
- Sample is discarded by incineration.

6.10. BODY FLUIDS

- Body fluids include CSF, peritoneal, pericardial, pleural, synovial fluid etc.
- Normally a very small amount of fluid is present in the body cavities.
- 1-3 ml of fluid is required.
- Can be stored by refrigeration up to 1 week.

- **Indications:**

CSF – Detection and diagnosis of meningitis. Cerebral infarction or hemorrhage Introduction of drugs, radiographic contrast media etc. **Peritoneal fluid** Ascites of unknown etiology, intra abdominal hemorrhage due to trauma, malignancies etc. **Pericardial fluid** Acute or chronic cardiac tamponade, pericardial effusion of unknown etiology etc.
Pleural effusion: - Effusion of unknown etiology, effusion of known etiology, intra pleural installation of drugs etc. Report will be available within 4 hours. Body fluids are discarded by incineration.

6.11. URINE

- Mid stream specimens may be taken

Collect urine directly into a sterile wide mouthed, screw-capped bottle.

- Specimens should be sent to the lab within 30 minutes of collection or refrigeration can be done but no longer than 1-3 hours.
- **Indications** – Urinary tract infections, diabetes, presence of protein, glucose, blood, ketone bodies along with microscopy can be assessed.
- Report – available in one hour to 6 hours depending on the test asked for.

6.12. BT/CT

- Ivy method and capillary tube method for BT and CT respectively.
- In patient done in ward, OP done in the lab.
- Done for evaluation of abnormal bleeding as a screening test.

Test Name	**Sample**	**Sample collection instructions**	**Test Method**	**Turnaround time**	**TAT for urgent tests**	**Clinical Utility**
A.E.C. (Absolute Eosinophil Count)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 hours	1 Hour	To assess the degree of eosinophilia.(500-1500 =mild; 1500-5000=moderate; >5000 =severe.)
Absolute Lymphocyte Count	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	1 Hour	To assess the degree of absolute lymphocytosis or lymphocytopenia
Absolute Neutrophil Count	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	1 Hour	To assess the degree of absolute neutrophilia or neutropenia.
BT (Bleeding Time) & CT(Clotting Time)	Blood	NA	BT – Duke method CT-capillary tube method	2 hours	30 mins.	Bleeding time is affected by platelet function, certain vascular disorders and von Willebrand Disease. Clotting time is the time required for a sample of blood to coagulatein vitro under standard conditions. Platelet count

						and PT is preferred over BT and CT .
Complete Blood Count (RBC,HB, TC,DC, PC, MCV, MCH, MCHC)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Used for diagnosis of anemia, infection, leukemias, clotting disorders etc.
Complete Haemogram (HB, TC,DC,ESR, RBC, PCV,MCV,MCH, MCHC, RDW, PC, PS)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	This test provides information about red cells, white cells and platelets. Results are useful in the diagnosis of anemia, infections, leukemias, clotting disorders and many other medical conditions. ESR acts as an acute phase reactant.
Erythrocyte Sedimentation Rate (ESR)	Blood	2 ml Blood citrated	Automated Cell Counter; westergreen	6 Hours	1 hour 30 mins	ESR is an acute phase reactant which indicates presence and intensity of an inflammatory process. It is never diagnostic of a specific disease. It is used to monitor the course or response to treatment of certain diseases. Extremely high levels are found in cases of malignancy, hematologic diseases, collagen disorders and renal diseases.
Haemoglobin (Hb)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Hemoglobin levels are used to diagnose type and degree of anemia. Several factors like age, sex, pregnancy and diurnal variations are to be considered before diagnosis of anemia is made on the basis of single hemoglobin estimation.
Leukocyte Count (Differential) (DC)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Major infection fighting cells. Also involved in reactions to

						allergies, tumor and stress in general
Leukocyte Count Total (TC)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Major infection fighting cells. Also involved in reactions to allergies, tumor and stress in general
Packed Cell Volume (PCV)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	In diagnosis of anemia
Peripheral Smear (PBS)	Blood	2 ml blood in EDTA and/or 2 blood smear slides	Microscopy	6 Hours	1Hour	Interpretation of Morphologic variation
Platelet Count	Blood	2 ml blood in EDTA and/or 2 blood smear slides	Microscopy/ Automated Cell Counter	6 Hours	1Hour	Aids in evaluating primary hemostasis
Red Blood Cell Count (RBC)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Aids In diagnosis of anemia and polycythemia.
Red Blood Cell Indices	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Gives MCV, MCH, MCHC and RDW. Aids in the evaluation of anemia
Reticulocyte Count (Retic)	Blood	2 ml EDTA Blood	Supra Vital stain on slide	6 Hours	2 Hour	Reticulocytes are produced during the process of erythropoiesis and are somewhat larger than mature erythrocytes. Reticulocyte count provides an initial assessment of whether the cause of anemia is due to impaired RBC production or due to increased loss in the peripheral circulation e.g. blood loss, hemolysis.
MCHC (Mean Corpuscular Hemoglobin Concentration)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Gives the mean concentration of the heamoglobin within the RBCs. MCHC is reduced in Iron deficiency anemia

						and increased in spherocytosis.
MCH(Mean Corpuscular Hemoglobin)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	Gives the average mass of hemoglobin per red blood cell in a sample of blood.MCH is reduced in Iron deficiency anemia.
MCV(Mean Corpuscular volume)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	To assess the RBC size. Helps classify anemias into microcytic, normocytic or macrocytic.
RDW (Red cell distribution width)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	To assess the degree of RBC anisocytosis.
MPV (Mean Platelet Volume)	Blood	2 ml EDTA Blood	Automated Cell Counter	6 Hours	2 Hour	-Gives the average size of platelets found in blood . -Can be used to make inferences about platelet production in bone marrow or platelet destruction problems.
Ascitic Fluid cell count and Cell Type	Ascitic fluid	2 ml Ascitic fluid in EDTA	Microscopy	6 Hours	3 Hour	For cell type and cell count
CSF Cell Count/Cell Type	CSF	2 ml CSF in EDTA container	Microscopy	6 Hours	3 Hour	Diagnosis of meningitis
Pleural Fluid Cell Count/ Cell Type	Pleural Fluid	2 ml Pleural fluid in EDTA container	Microscopy	6 Hours	3 Hour	Aids in assessment of the type of effusion
Synovial Fluid	Synovial Fluid	Synovial fluid in plain and EDTA container (sterile)	Microscopy	6 Hours	3 Hour	Aids in assessment of the type of effusion
Pericardial fluid	Pericardial fluid	Pericardial fluid in plain and EDTA container (sterile)	Microscopy	6 Hours	3 Hours	Aids in assessment of the type of effusion

Routine (Urine Complete)	Urine	Spot urine sample in leak proof container	Biochemical Microscopy	6 Hours	1 Hour	Helps screening UTI and renal diseases
Urine SG-Specific Gravity	Urine	10ml urine sample in leak proof container	Biochemical	6 Hours	1 Hour	To assess the urine concentrating capacity of the renal tubules.
Urine pH	Urine	Morning urine sample in leak proof container	Biochemical	6 Hours	1 Hour	The test measures the level of acid in urine.
Sugar (Urine)	Urine	Morning urine sample in leak proof clean container	Biochemical	6 Hours	1 Hour	This test is most commonly used to screen for possible diabetes
Urine Bile Salt	Urine , spot	20 ml of spot urine	Dipstick	6 Hours	1 Hour	Used for patients who show signs of abnormal liver function
Urine Bile Pigment	Urine , spot	20 ml of spot urine	Dipstick	6 Hours	1 Hour	Used for patients who show signs of abnormal liver function
Protein Urine qualitative	Urine	20ml, random	Dipstick	6 Hours	1 Hour	Urinary total proteins are negligible in healthy individuals. Levels are increased in diseases that impair renal function like Diabetes, Hypertension, Nephrotic Syndrome and drug nephrotoxicity
Urine Ketone Bodies	Urine , spot	10 ml of spot urine sample	Dipstick	6 Hours	1 Hour	Ketonuria
Urine Blood (Hb/ Hb derivative)	Urine , spot	20 ml of spot urine sample	Dipstick	6 Hours	1 Hour	The presence of blood in urine may be associated with a urological cause, a renal cause/ be due to a UTI.
Urine for Fat Globules	Urine	20ml, random	Microscopy	6 Hours	1 Hour	Lipiduria
Urobilinogen	Urine	10 ml of spot urine sample	Dipstick	6 Hours	1 Hour	Test is used to assist a diagnosis of acute intermittent porphyria