STANDARD OPERATING PROCEDURE
GENERAL LABORATORY SAFETY PROCEDURES & RULES

Document ID Code: 100 *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Laboratory safety

All students/staff must read and understand the information in this document with regard to laboratory safety and emergency procedures prior to the first laboratory session. **Your personal laboratory safety depends mostly on YOU**. Effort has been made to address situations that may pose a hazard in the lab but the information and instructions provided cannot be considered all-inclusive.

Students/staff must adhere to written and verbal safety instructions throughout the academic/working term. Since additional instructions may be given at the beginning of laboratory sessions.

With good judgment, the chance of an accident in this course is very small. Nevertheless, research and teaching workplaces (labs, shops, etc.) are full of potential hazards that can cause serious injury and or damage to the equipment. Working alone and unsupervised in laboratories is forbidden if you are working with hazardous substances or equipment. With prior approval, at least two people should be present so that one can shut down equipment and call for help in the event of an emergency.

Safety training and/or information should be provided by a faculty member, teaching assistant, lab safety contact, or staff member at the beginning of a new assignment or when a new hazard is introduced into the workplace.

Emergency Response

1. It is your responsibility to read safety and fire alarm posters and follow the instructions during an emergency
2. Know the location of the fire extinguisher, eye wash, and safety shower in your lab and know how to use them.
3. Notify your instructor immediately after any injury, fire or explosion, or spill.
4. Know the building evacuation procedures.
5. ***Common Sense***

Good common sense is needed for safety in a laboratory. It is expected that each student will work in a responsible manner and exercise good judgement and common sense. If at any time you are not sure how to handle a particular situation, ask your Teaching Assistant or Instructor for advice. **DO NOT TOUCH ANYTHING WITH WHICH YOU ARE NOT COMPLETELY FAMILIAR**!!! It is always better to ask questions than to risk harm to yourself or damage to the equipment.

Personal and General laboratory safety

1. Never eat, drink, or smoke while working in the laboratory.
2. Read labels carefully.
3. Do not use any equipment unless you are trained and approved as a user by your supervisor.
4. Wear safety glasses or face shields when working with hazardous materials and/or equipment.
5. Wear gloves when using any hazardous or toxic agent.
6. Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time.
7. If you have long hair or loose clothes, make sure it is tied back or confined.
8. Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment that requires air flow or ventilation to prevent overheating.
9. Disposal - Students are responsible for the proper disposal of used material if any in appropriate containers.
10. Equipment Failure - If a piece of equipment fails while being used, report it immediately to your lab assistant or tutor. Never try to fix the problem yourself because you could harm yourself and others.
11. If leaving a lab unattended, turn off all ignition sources and lock the doors.
12. Never pipette anything by mouth.
13. Clean up your work area before leaving.
14. Wash hands before leaving the lab and before eating.

Electrical safety

1. Obtain permission before operating any high voltage equipment.
2. Maintain an unobstructed access to all electrical panels.
3. Wiring or other electrical modifications must be referred to the Electronics Shop or the Building Coordinator.
4. Avoid using extension cords whenever possible. If you must use one, obtain a heavy- duty one that is electrically grounded, with its own fuse, and install it safely. Extension cords should not go under doors, across aisles, be hung from the ceiling, or plugged into other extension cords.
5. Never, ever modify, attach or otherwise change any high voltage equipment.
6. Always make sure all capacitors are discharged (using a grounded cable with an insulating handle) before touching high voltage leads or the "inside" of any

equipment even after it has been turned off. Capacitors can hold charge for many hours after the equipment has been turned off.

7. When you are adjusting any high voltage equipment or a laser which is powered with a high voltage supply, USE ONLY ONE HAND. Your other hand is best placed in a pocket or behind your back. This procedure eliminates the possibility of an accident where high voltage current flows up one arm, through your chest, and down the other arm.

Mechanical safety

1. When using compressed air, use only approved nozzles and never directs the air towards any person.
2. Guards on machinery must be in place during operation.
3. Exercise care when working with or near hydraulically- or pneumatically-driven equipment. Sudden or unexpected motion can inflict serious injury.

Chemical safety

1. Treat every chemical as if it were hazardous.
2. Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
3. Never return chemicals to reagent bottles. (Try for the correct amount and share any excess.)
4. Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc. If uncertain about regulations, contact the building coordinator.
5. Use volatile and flammable compounds only in a fume hood. Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material.
6. Never allow a solvent to come in contact with your skin. Always use gloves.
7. Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents.
8. Dispose of waste and broken glassware in proper containers.
9. Clean up spills immediately.
10. Do not store food in laboratories.

Lasers safety

1. NEVER, EVER LOOK INTO ANY LASER BEAM, no matter how low power or "eye safe" you may think it is.
2. Always wear safety goggles if instructed by your Instructor or Teaching Assistant.
3. The most common injury using lasers is an eye injury resulting from scattered laser light reflected off of mountings, sides of mirrors or from the "shiny" surface of an optical table. The best way to avoid these injuries is to always wear your goggles and NEVER LOWER YOUR HEAD TO THE LEVEL OF THE LASER BEAM! The laser beam should always be at or below chest level.

4. Always use "beam stops" to intercept laser beams. Never allow them to propagate into the laboratory. Never walk through a laser beam. Some laser beams of only a few watts can burn a hole through a shirt in only a few seconds.
5. If you suspect that you have suffered an eye injury, notify your instructor or teaching assistant IMMEDIATELY! Your ability to recover from an eye injury decreases the longer you wait for treatment.

Additional Safety Guidelines

- Never do unauthorized experiments.
- Never work alone in laboratory.
- Keep your lab space clean and organized.
- Do not leave an on-going experiment unattended.
- Always inform your instructor if you break a thermometer. Do not clean mercury yourself!!
- Never taste anything. Never pipette by mouth; use a bulb.
- Never use open flames in laboratory unless instructed.
- Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates.
- Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes.
- Do not use corridors for storage or work areas.
- Do not store heavy items above table height. Any overhead storage of supplies on top of cabinets should be limited to lightweight items only. Also, remember that a 36" diameter area around all fire sprinkler heads must be kept clear at all times.
- Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly. However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present.
- Be careful when lifting heavy objects. Only shop staff may operate forklifts or cranes.
- Clean your lab bench and equipment, and lock the door before you leave the laboratory.

Safe Laboratory Practices & Procedures

Below is a listing of Safe Laboratory Practices to serve as a reminder of some fundamental safety tips.

1: Ask yourself, "What am I working with? What are the hazards?"

- Common hazards in the laboratory include: animal, biological, chemical, physical, and radiological. If there is an accident or emergency situation involving these hazards:
    - Seek immediate assistance. If you are splashed by any of these materials, use running water from an eyewash station or emergency shower for at

least 15 minutes or until emergency assistance arrives and provides you with different instructions.
    - Report to your supervisor any accident, injury, or uncontrolled release of potentially hazardous materials - no matter how trivial the accident, injury, or release may appear.

2: Be prepared.

- Attend all required laboratory safety training prior to the start of your research assignment.
- Read all procedures and associated safety information prior to the start of an experiment.
- Perform only those experiments authorized by your supervisor.
- Follow all written and verbal instructions. Ask for assistance if you need guidance or help.
- Work under direct supervision at all times. Never work alone in the laboratory.
- Know the locations and operating procedures for all safety equipment. This includes the eyewash station and safety shower.
- Know the locations of the nearest fire alarms and at least two ways out of the building. Never use an elevator in emergencies.
- Be alert and proceed with caution at all times in the laboratory. Immediately notify the supervisor of any unsafe conditions.
- Know the proper emergency response procedures for accidents or injuries in the laboratory.

3: Prevent potential exposure.

- Conduct yourself in a responsible and professional manner at all times. No pranks. No practical jokes.
- Dress for work in the laboratory. Wear clothing and shoes that cover exposed skin and protect you from potential splashes. Tie back long hair, jewelry, or anything that may catch in equipment.
- Never eat food, drink beverages, chew gum, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- Use a chemical fume hood or biosafety cabinet, as directed by your supervisor.
- Observe good housekeeping - keep aisles clear.
- Report damaged electrical equipment to the supervisor. Do not use damaged electrical equipment.
- Do not leave active experiments unattended. Never leave anything that is being heated or is visibly reacting unattended.

4: Protect yourself, others, your research, and the environment.

- Practice good personal hygiene. Wash your hands after removing gloves, before leaving the laboratory, and after handling a potentially hazardous material.
- While working in the laboratory, wear personal protective equipment - eye protection, gloves, and laboratory coat - as directed by your supervisor.

- Properly segregate and dispose of all laboratory waste.

REFERENCE:

Biosafety and Biosecurity draft manual

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
SPECIMEN COLLECTION

Document ID Code: 100 *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Valid laboratory results are dependent upon proper specimen collection and handling prior to the arrival of the sample in the laboratory. The following is a basic phlebotomy procedure, followed by procedures specific for each type of collection.
Note:
There are business specific and in-house requirements for filling out Requisition/consent forms and for collections. Refer to appropriate department/unit Appendix and/or procedures.

PHLEBOTOMY

1. Wash hands thoroughly before beginning any phlebotomy procedure. Be sure to check expiration dates on tubes before proceeding. DO NOT USE EXPIRED TUBES.
2. Confirm the identity of the patient by checking at least two identifiers before collecting the specimen(s). This can be done by asking the patient to state their full name and requesting to see the patient's driver's license to verify picture, name, date of birth and/or driver's license number and documenting the information on the consent/chain of custody form.
3. Explain the procedure, including small risk of hematoma, slight pain, and some light-headedness. Inquire whether the patient has a history of fainting or dizziness with phlebotomy procedures so that ammonia inhalants can be obtained if necessary. Explain that loss of vacuum or a collapsed vein may necessitate another draw.
4. On a table or desk, assemble all necessary equipment: cotton balls and/or gauze, tubes, safety needle, alcohol swab, tourniquet, gloves, and band aid. Wearing safety gloves is MANDATORY. Wear additional protective equipment if contamination is expected. Safety needles should always be used; the only exception is if the patient is very hard to draw then a butterfly needle set may be used.
5. Position the patient so that they are seated comfortably in a chair with their arm extended on an armrest, desk, or table to form a straight line from the shoulder to the wrist. The patient's arm and elbow should be firmly supported, and not bent at the elbow.

6. Check both arms to select the larger and fuller veins. Palpate and trace the path of the veins several times with your index finger. Tap the vein at the site of the draw with your index finger and second finger. This will cause the vein to dilate.

The following factors should be considered in site selection:

i) Extensive scarring. Healed burn areas or scar tissue should be avoided.

ii) Specimens collected from an area with a hematoma may yield erroneous test results. If another vein site is not available, the specimen should be collected distal to the hematoma.

7. Apply the tourniquet.

8. Ask the patient to open and close his/her fist so their veins become prominent. Vigorous hand pumping is not necessary to activate blood flow and should be avoided.

9. Clean the venepuncture site with the alcohol swab in a circular motion from the centre of the area to the outside. Allow the area to air-dry to prevent haemolysis and a burning sensation to the patient.

10. Insert the stopper of the first tube to be drawn into the adaptor. Do not push too far to avoid premature loss of vacuum via puncture of the needle.

The recommended order of draw when drawing more than one tube is as follows:

♦Non additive tube (red stopper)

♦Coagulation tube (light blue stopper)

♦Serum separator tube (SST) or serum tube

♦Additive tube (lavender stopper, green stopper, etc

11. Insert the needle into the vein with the bevel facing upward. Puncture the stopper on the tube by pushing it onto the end of the needle, and grasp the edge of the Adaptor to provide stability once the blood flow has begun. Have the patient open his/her fist.

12. Fill the tube until the vacuum is exhausted. Remove the tube from the adaptor and insert subsequent tubes. Be sure that all tubes are completely filled to ensure sufficient blood sample for laboratory analysis.

13. Place a cotton ball or 2 x 2 square piece of gauze over the site. All used needles must be disposed of in a puncture proof biohazard receptacle. Never recap a needle. Recapping, purposeful bending, breaking, removing from disposable syringes, or other manual manipulations of needles is prohibited. Apply pressure to the site for 2-5 minutes. Place a band aid over the puncture site.

14. Again verify that the information on the sample tubes match the consent/requisition form.

15. Remove gloves and dispose of in a properly identified biohazard bag or container. Wash hands thoroughly after phlebotomy.

ADDITIONAL VENIPUNCTURE CONSIDERATIONS

1. Prevention of Hematoma:

a) Puncture only the uppermost wall of the vein

b) Release the tourniquet before removing the needle from the vein.

c) Use only major veins; not superficial veins

d) Make sure that the needle fully penetrates the uppermost wall of the vein. Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel.

e) Apply a small amount of pressure to the area with the cotton ball or gauze pad when bandaging the arm.
2. Prevention of Hemolysis:
a) Mix anticoagulated specimens thoroughly by inverting each tube gently 8 to 10 times. Do not shake. Vigorous mixing may cause hemolysis.
b) Avoid drawing blood from an area with a hematoma.
c) Ascertain that the venipuncture site is dry without touching it.
3. If a Blood Sample is Unobtainable
a) Change the position of the needle. If the needle has penetrated too far into the vein, pull it back slightly. If it has not penetrated far enough, advance it farther into the vein. Rotate the needle a half-turn.
b) Try another tube; the tube may not have sufficient vacuum.
c) Loosen the tourniquet. It may be applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. This procedure can be accomplished easily when using the Velcro-type tourniquet by releasing it and quickly pressing it together again.
d) Probing for the vein is NOT recommended as it is painful to the patient. In most cases, another puncture in a site below the first site is advised
e) Never attempt a venipuncture more than twice. Have another person attempt to draw the specimen.

SPECIMEN HANDLING

1. Gently invert SST tubes 5 times and all tubes with anticoagulant (EDTA, heparin, etc.) 8 to 10 times.
2. Ensure all tubes are labelled with identification number and second identifier (printed first and last name for Insurance applicants).
3. Let red-top and marbled tubes clot, preferably in an upright position, for 30 minutes, but not more than 45 minutes. Centrifuge the tube for 5-10minutes at 2500-3500 rpm and transfer the serum into a properly labelled pour-off tube using a disposable pipette.
4. Some other factors that can affect the sample are:
a) **Hemolysis.** Hemolysis is defined as the breaking down of red blood cells. This can be slight, moderate or severe. The three (3) causes of Hemolysis are TIME, TEMPERATURE AND TRAUMA.
i) **TIME**: Holding blood over two (2) hours before centrifuging can and usually does cause some Hemolysis. Allow at least 10-20 minutes, but no more than 45 minutes for the blood to clot prior to centrifuging.

ii) **TEMPERATURE**: Never store blood in too warm an area; hot cars, hot sun, etc. Allowing blood to freeze in cold weather will also produce hemolysis.
iii) **TRAUMA**: Going through the vein, accessing a collapsed vein, or using a needle that is too small can all cause hemolysis. The needles provided by the labs are usually 21 or 22 gauge. If a 23 gauge is used, it is very possible that hemolysis may occur. Only use a smaller needle when absolutely necessary. When doing fingersticks, DBS, etc., squeezing the finger is the main cause of hemolysis.

b) **Lipemia:** Lipemia is defined as an abnormal amount of fat in the blood. This is usually caused by the patient not fasting.

REFERENCES

- Ministry of Health SOP manual, sample collection and handling reference file.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: alex t *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
GRAM STAIN SOPS.

Document ID Code: 100 *Version No. 1.0*

Effective Date: 01/02/2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST.

Serum positive organisms retain crystal violet colour hence staining dark purple due to presence of thick glycol-protein layer while gram negative organisms are decolorized by acetone due to thin layer of glycol-protein layer.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST.

Help to distinguish between gram positive and gram negative organisms for better medication.

EQUIPMENT.

- Microscope.
- Staining rack.
- Dry rack.

EQUIPMENT MAINTENANCE SCHEDULE.

Regular micro-scope cleaning with xylene and regular 3 months service.

REAGENTS AND MATERIALS.

Crystal violet, grams iodine, acetone, neutral red, staining rack, drying rack and swabs.

REAGENT PREPARATIONS.

1) Gram stain
   - Crystal violet – 0.5g
   - Distilled water – 100ml.
2) Neutral red
   - Neutral red – 1g
   - Acetic acid 1% - 2ml
   - Distilled water – 1000ml.
3) Lugol's iodine
   - Iodine – 10g
   - Potassium iodine – 20g
   - Distilled water – 1000ml.
4) Acetone

SAMPLE REQUIRED – Pus Swab, HVS, Cervical swab, Urethral Swab.
SAMPLE COLLECTION – Sterile swabs.

PROCEDURES.

- Prepare a smear, allow to air dry and fix with gentle heat.

- Apply crystal violet and allow staining for 1 minute.
- Wash with distilled water.
- Replace with Lugol's iodine and allow staining for 1 minute.
- Wash with distilled water.
- Replace with acetone until no more colour appears to flow from the preparation.
- Wash with water.
- Apply neutral red and allow staining for 3-4 minutes.
- Rinse with water.
- Dry and examine with oil immersion x100 objective.

REPORTING AND INTERPRETATION OF RESULTS.

- Gram +ve – Dark blue.
- Gram –ve – Purple.

Internal QC

Trouble shooting/source of error.

(1) Overturning with any stain i.e. crystal violet.
(2) Over decolorizing with acetone.
(3) Dirty microscope objective x100.

SAFETY PRECAUTIONS.

- Wear gloves and masks when doing the procedure.

REFERENCE.

Medical Laboratory Technology by T.J Baker

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by:alex t *Signature:* ....

Approved by: *Signature:* ………………………..

****

STANDARD OPERATING PROCEDURE
STOOL ANALYSIS.

Document ID Code: *Version No. 1.0*
Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019
Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE

It is based on receiving many substances and organisms and parasites i.e. Entamoeba histolytica, giardia lamblia. Ascaris lumbricoide, hook worms etc and substances like, crystals, fats, etc.

Purpose of the test/clinical significance; To diagnose for parasite infestation.

Equipment – Microscope

Equipment maintenance – schedule regular cleaning.

Reagents and materials – normal saline, microscope slides, cover slips, applicator Sticks, polypot.

Sample Required – stool.

Sample collection – Done by the patient at the wash room.

Reagent preparation – physiological saline.

- 0.8gm of sodium chloride.
- 1 little distilled water.

PROCEDURE

1) Place one drop of saline on one and of a microscope slide.
2) Place one drop of lugols iodine at the other end.
3) Emulsifying a small portion of stool in the saline using applicator stick in both reagents.
4) Apply a cover slip to each.
5) Examine with x10 to see the field and then x40 to see the details.

- ***Reporting and Interpretation.***

  Report any oval, crystal or Trophozoite seen also report any other abnormality detected i.e. fat glucose, pus cells etc.

Trouble shooting/any source of error.

Dirty microscope lenses.

- ***safety precautions***

  Wear protective gloves and masks always when handling specimen.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
URINE ANALYSIS SOPS.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE

It is based on the qualitative and quantitative detection and estimation of various substances in a specimen or urine.

PURPOSE OF THE TEST – To detect and estimate various substance in urine specimen i.e. glucose, protein, PH. Etc.

EQUIPMENT: Combilyzer plus, microscope
EQUIPMENT MAINTENANCE SCHEDULE – three months intervals
REAGENTS AND MATERIALS – Urine test strips, centrifuge tubes, urine bottles, glass slides and cover slips
REAGENT PREPARATION – Readily available from manufacture.
SAMPLE REQUIRED – Urine (first morning) (MSU).
SAMPLE COLLECTION – patients are advised to put MSU.

PROCEDURE

1) Collect specimen in clean, well rinsed container free from detergents, preservation and disinfectants.
2) Transfer well mixed uncentrifuged urine into a centrifuge tube.
3) Remove the test strip from the container and immerse immediately into the tube containing urine for approximate 1-2 seconds.
4) Remove excess urine from the strip by wiping the edge of the strip on the tube.
5) Place the strip on the test area of the machine and press the start button.
6) Centrifuge the remaining sample at 1,500rpm for 3 minutes discard the supernatant and place one small drop of the loosened deposit on a clean glass slide, cover the drop with a cover slip gently and examine under the microscope using x10 objective to see the field and the x40 objective to distinguish the morphology.

REPORT.

REFERENCE

1) A hand book of clinical chemistry for laboratory technicians by sitati.
2) A hand book of medical laboratory practices part 1 by Monicah Cheesebrough.

<u>INTERNAL QC</u> – Using normal saline.
TROUBLE SHOOTING/SOURCES OF ERROR – Regular washing of the strip carrion plus calibration using a new kit code.

<u>INTERPRETATION OF RESULTS</u>- Colour change of different parameters is compared from the original one i.e. Glucose with yellow changes according to the concentration of glucose in urine to different colours code i.e. light green –deep green.

<u>SAFETY PRECAUTIONS</u> – Wearing of latex gloves when performing the procedure always.

<u>APPOVAL.</u>
The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: ....*

Approved by: *Signature: …………………….....*

****

STANDARD OPERATING PROCEDURE
WEAK ANTI-D TEST. (DU TEST)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test: Patients cells are exposed to known anti-D serum to establish the Rhesus factor on the patient cells.
Clinical significance/purpose of the test: To confirm a true rhesus negative.
Equipment: Water bath, centrifuge, microscope.
Equipment maintenance schedule: Routine service as per hospital policy on duration of 3 months, regular cleaning.
Reagent preparation: Ready to use reagent is supplied (Anti-D and anti-human globulin).

Normal Saline (0.85% Nacl)

- Dissolve 0.85 of sodium chloride in 100ml distilled water.

Reagent and materials: Anti-D, AHG, glass slide, test tubes, normal saline.
Sample required: Blood in EDTA Tube.
Sample collection:

- Select the best vein.
- Tie the toniquet.
- Swab the vein with spirit swab.
- Withdraw about 3mls of blood.
- Sample is ready for analysis

Procedure:

- Label a tube Du.
- Put equal volumes of patient cells and anti –D grouping sera.
- Incubate at 37°c for ½ - 1 hour.
- Wash the tube with saline 3 times.
- Add 1 volume of A.H.G and spin for 1 min. at 1000 rpm.
- Check for agglutination by gently tilting the tube and then confirm by the use of microscope x40.
- Report Du positive or negative.

Reporting and interpretation of results.

- If agglutination occurs report Du positive.
- If no agglutination occurs report Du negative hence rhesus negative.

Internal Quality Control.

- Using a known rhesus negative known sample.

Trouble shooting/source of error.

- Using diet microscope slides and tubes.

Safety Precautions

- Make sure you have put anti-seras.
- Use patients 4% cells suspension.
- Wear protective gloves throughout the procedure.

References.

- Medical Laboratory practices part 2.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
BRUCELLIN TEST.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principles of the test: The smooth attenuated stained antigen suspensions are mixed with the patient's serum. Specified antibodies to Brucella antigens if present in the patient serum will react with the antigen suspensions to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to Brucella antigens.

Clinical significance

- Brucella test is carried out to diagnosis the patient for brucellosis (undulant fever).
- Brucella species are detectable a few weeks after exposure and are of considerable importance in the diagnosis of brucellosis.

Equipment; Centrifuge machine, Rotator.
Equipment maintenance schedule: Both centrifuge machines and rotators are serviced twice in a year, on June and December.
Reagents Required: Brucella mellitensis and Brucella Abortus Antigen suspensions.
Materials Required: Applicator sticks, test cards, stopwatch, appropriated pipette, high intensity direct light source.

Reagents Preparations: Commercially available.
Sample Required: Freshly collected sample and non-haemolysed.
Sample Collection

- Select the best vein to collect adequate amount of blood
- Sterilize with 70% alcohol at the site to make vein puncture.
- Tie the toniquet.
- Using a vacutainer needle and tube with clot activator or with red cap collect about 3ml of blood.
- Allow blood to clot properly.
- Centrifuge to separate serum and cells with 4000 revolution per min for 3 minutes.
- Serum is ready to be tested.

Procedure.

Slide test method (Semi-quantitative method)

- Using a pipette place 80ul, 40ul, 20ul, 10ul and 5ul of the patient serum to be tested on 5 different circles on the test cards. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160 and 1:320 respectively.
- Place one drop of appropriate antigen suspensions to each circle.
- Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
- Gently rotate the test cards with contents, observe for agglutination macroscopically at one minutes against a white back ground.

Reporting and interpretation of the results

Agglutination is a positive test result. The titres of patient serum correspond to the visible agglutination in the test circle with minimum amount of serum sample.

Internal Quality Control

Using provided positive and negative control.

Sources of error

- Using expired Antigen suspensions.
- Using haemolysed samples.
- Using tubes containing any kinds of anticoagulant.
- Using expired test tubes and not properly clotted serum.

Safety Precautions

- Always wear gloves when performing any test.
- Incase of sample spillage on the bench ensure you decontaminate the bench using sodium hypochlorite.

References: Monicah Cheesebrough. Volume 1

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE.
SALMONELLA "O" ANTIGEN AND SALMONELLA "H" ANTIGEN FOR WIDAL TEST.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test:

When the coloured, smooth suspension of attenuated antigen suspensions is mixed with patient serum anti-salmonella antibodies present in the patient serum react with the antigen suspension to produce agglutination. Agglutination is a positive test result, indicating presence of salmonella antibodies in the patient sample.

Clinical Significance:

To detect antibodies produced in response to the stimulation by specific antigens of salmonella group.
The test is also used to monitor the effectiveness of the treatment.

Equipment: Centrifuge, Mechanical Rotator set at 100 r.p.m.
Equipment maintenance schedule:
Lubricate the machine (both) after 3 months.
General services done twice a year.

Reagent and Materials

Reagents: Antigen suspension salmonella typhi: "O".
Antigen suspension salmonella typhi "H".
Polyspecific positive control (goat).
Materials: Applicator sticks.
Pasteur pipettes.
Stop watch.
Test card/clear glass slide.
Micro pipette (adjustable).

Reagent preparation: All reagents are commercially available.
Sample Required: Fresh serum Stable for 7 days at 2 – 8° C.
Sample s should be free from contamination, hemolysis and Lipemia.

Sample Collection.

- Collect 3ml of patient blood in a tube containing clot activator or plain tube.
- Allow the blood to clot properly and then centrifuge to get adequate serum.
- Avoid haemolysed blood sample always.

Procedure (Semi – quantitative method).

1. Pipette one drop of antigen into the first reaction circle and then place 5, 10, 20, 40, 80 ul of the test sample on the remaining circles.

2. Resuspend the antigen slightly then add to each reaction circle a drop of the antigen which showed agglutination with the test sample in the screening method.
3. Using a mixing stick, mix the contents of each circle uniformly over the reaction circles.
4. Mixing the contents on the reaction circles for one minute using the rotator.

Reporting and interpretation of the results.

Titre of the antibody is given by the last reaction circle showing agglutination and they correspond to the following

Antibody titre	Serum volume u/l
1:20	80
1:40	40
1:80	20
1:160	10
1:320	5
1:640	2.5

Agglutination with 10ul of test serum i.e. 1:160 antibody title usually suggest infection.

Internal Quality Control.

Positive and negative controls are run at least twice in a week to validate the result. Both are bought when purchasing Widal test kit.

Sources of Error.

- Using expired antigen "O" & "H" suspension.
- Using haemolysed blood sample.
- Using blood/serum which is not fully clotted.
- Using lipemic samples.

Safety Precautions.

- Always use gloves when handling and analysing human blood sample.
- Always decontaminate the bench using sodium hypochlorite in case you spill patient's blood/serum sample.

Reference: Monicah Cheesebrough volume 1

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
H. PYLORI.

Document ID Code: *Version No. 1.0*
Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019
Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test:

H.pylori rapid test strip (whole blood/serum plasma) is a qualitative membrane strip based immunoassay for the detection of H.pylori antibodies in whole blood, serum or plasma. In this test procedure the test strip immersed in the specimen or specimen containing buffering solution. This specimen migrates chromatographically along the length of the test strip. If the specimen does not contain H.pylori antibodies a coloured line will not appear in this region indicating a negative result.

Clinical Significance:

The H.pylori rapid test strip is a rapid chromatographic immunoassay for the qualitative detection of antibodies to H.pylori in whole blood, serum or plasma to aid in the diagnosis of H.pylori infection.

Equipment: Centrifuge, Stop watch.

Equipment maintenance schedule:

Lubricate movable parts with grease after every three months and general services done twice a year.

Reagent preparation: Commercially available test strips.

Sample Required: Whole blood/serum /plasma.

Material required

- Specimen collection container.
- Needle (Vacutainer needle).
- Needle holder.
- Disposable heparinised capillary tubes and dispensing bulb (for finger prick).
- Lancet (for finger stick whole blood only).
- Dropper (adjustable pipette).

Blood collection procedure (vein puncture).

- Sterilize the site to make vein puncture using sprint swab.
- Select best vein to make puncture.
- Collect sufficient amount of blood sample using vacutainer needle and the tube Containing or coated with serum activator.
- Allow the sample to clot properly.
- Centrifuge the blood and separate serum from other blood cells.
- The serum is now ready to be tested.

To collect finger stick whole blood specimens.

- Massage the hand without touching the puncture site by rubbing down the hand towards the fingertips of the middle or ring finger.
- Puncture the skin with sterile lancet, wipe away the first sign of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.

- Add the finger stick whole blood specimen to the test strip using a capillary tube approximately 50ul.
- Place the bulb on the top end of the capillary tube then squeeze the bulb to disperse the whole blood onto the specimen pad of the test strip.
- Whole blood collected by finger prick should be tested immediately.

Procedure of the test.

- Remove the test strip from the foil punch and use it as soon as possible.
- For serum or plasma specimen. Hold the dropper vertically and transfer 4 drops of serum/plasma (approx 100ul) on the specimen pad then start the timer.
- For finger prick whole blood specimen: To use a capillary tube fill the capillary tube and transfer approximately 50ul of finger prick whole blood specimen on to specimen pad of test then add 1 drop of buffer (aprox 40ul and start the timer.)
- Wait for the red line (s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

Interpretation of results.

Positive – Two distinct red lines appear.
Any shade of red in the test region (T) should be considered positive.

Negative – One red line appears in the central region ©.
No apparent red or pink line appears in the test region (T).

Invalid – Control line fail to appear.

Internal Quality Control.

Internal procedure controls are included in the test.
A red line appearing in the control region © is an internal positive control.

Trouble shooting

In case control line fails to appear may be to the following reasons.

- In sufficient specimen volume or in correct procedure technique are not followed properly.
- Review the procedure and repeat the test with a new test strip.

Safety precautions.

- Don't use strips after expiration date.
- Wear protective cloth such as laboratory coats.
- Always use disposable gloves.

References: Monicah Cheesebrough volume 2.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
BLOODSLIDE FOR MALARIA PARASITE USING GIEMSA STAIN.

Document ID Code: *Version No. 1.0*

Effective Date: 01.05.2017 *Revision Date: 01.05.2017*
Next Revision Date: 02.05.2018

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principles of the test: When a blood slide is stained by Giemsa, which is alcohol, based Romanosky stain it gives best staining of Malaria parasite in thin films and thick films.

CLINICAL SIGNIFICANCE

To diagnosis malaria parasite.

Equipment: Microscope, Staining rack, drying rank.

Equipment maintenance: Regular cleaning x 100 objective with xylene/general Service after 6 months, oil immersion, 70% alcohol, Pickers' EDTA tubes, cotton wool.

Reagent Preparation:

Solution 1: Methyl alcohol, absolute (alcohol), Giemsa stain.

Solution 2: Azure 11- eosin 3g.
Azure 11 8g.
Glycerol, pare 200ml.
Methyl alcohol, absolute (alcohol) 300ml.
Buffer solutions (PH 7.0)

Solution 3: Disodium hydrogen phosphate Na2Hpo4- 61.1ml.
Potassium dihydrogen phosphate KH2po4-38.9.

Dissolve the phosphate in the distilled water, check the reaction PH 7.0 and label.

Sample required: Whole Blood.

Sample Collection and Staining.

Procedures

- Place a completely clean (grease – free) and scratch free on the bench.
- Cleanse the finger using a swab moistened with 70% v/v alcohol.
- Using a sterile lancet prick the finger squeeze gently to obtain a large drop of blood.
- Collect the blood into small plastic bulb pipette.
- Dispense the blood to fill the large circle of the thick film and the small circle of the thin film.
- Immediately spread the film using a smooth edge spreader.
- Label the slide using a black lead penal including the plate and the patient's name and number.

- Allow the blood film to air dry with slide in a horizontal position and placed in a safe place.
- Fix thin smear with absolute methanol and allow film to fix for 1-2 minutes.
- Place the slide, smear downwards in a dish supported on each side by a thin piece of stick.
- Pour the diluted stain into the dish and cover with lid.
- Leave the smear to stain for 10 minutes.
- Wash the stain from the dish and rinse the smear with buffered water.
- Wipe the back of the slide and place it in a draining rack for smear to air-dry.
- Examine the smear microscopically with x40 objective to see the distribution of materials and examine with x100 objective for identification of parasite.

Reporting and Interpretation

Blood slide positive – report as follow.

No. of parasite counted against 200 white blood cells, multiplied by total white blood cells counted.

$$\frac{\text{No. of parasites counted x total white blood cells counted}}{200}$$

E.g. $\frac{20 \times 8000}{200}$ =800 parasite /ul

With thin film identify Malaria species and stage of development as follow:

- Plasmodium falciparum
- Plasmodium ovale

Internal Quality Control Procedure

- Known blood slide with both positive and negative Malaria parasites are prepared, fixed and stored well covered at freezer.
- They are used for controlling new prepared reagents before being used.

Trouble Shooting

- Microscope stage is cleaned daily with xylene to remove oil immersion.
- Objectives lenses are wiped with soft tissue daily before use.

Safety Precautions

- Always wear disposable gloves when carrying any procedure.

References:

- Monicah Cheesebrough volume 1 and part 2
- Eighth edition practical haematology by Sir John V. Docle S. M Lewis.
- Introduction to medical laboratory technology fifth edition by F.J Baker F.I.M.L.S, F.I.ST. And R.E. Silverton F.I.M.L.S, LIBiol.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
ZN STAINING FOR AFB.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test
When Mycobacterium are stained with carbol fuchsin in the ZN technique, they are able to retain (hold fast) heir red colour when washed with an acid solution. The acid fastness of mycobacterium is due to their thick cell wall which is composed of waxes and lipids that have a high content of mycolic fatty acids.

Purpose of the test.
Aids in the identification of mycobacterium tuberculosis which is the causative agent of tuberculosis infection.

Equipment.
- *X 100 objective microscope.*
- *Staining rack.*

Reagent and materials.
- 1% carbol fuchsin.
- 0.1% methylene blue.
- 25% sulphuric acid.
- Staining rack.
- Applicator sticks.
- Spirit swabs.
- Sputum containers.
- Drying rack.
- 5% phenol solution/5% sodium hypochlorite solution.
- Forceps.
- Oil immersion.

Reagent Preparation.

1% CARBOL FUCHSIN	***PREPARATION***
• contains per litre	dissolve phenol in alcohol in a 1 litre flask
• basic fuchsin 10gms	dissolve the fuchsin completely in the mixture
• Phenol crystals 50gms	add water to make 1 litre of solution, mix well.
• 95% ethanol/methanol	label: name of reagent, date of preparation, Signature of staff preparing.

- Water up to 1 litre.

0.1% METHYLENE BLUE ***PREPARATION***

Contains per litre

- Methylene blue powder 1.0gm
- Water 1 litre

Add 500mls of water to a litre flask
Add the methylene blue and mix until Completely dissolved.
Add remaining water to make 1 litre, Mix well
Label: name of reagent, date of Preparation, signature of staff preparing.

25% SULPHURIC ACID (Decolourising solution)

PREPARATION

Contains per litre

- Conc. Sulphuric acid 250ml
- Water 750ml

Add 750ml of water to a litre flask
Add acid slowly pouring down the The side of the flask
Stop and swirl flask regularly as a lot of Heat is generated until all the acid is Added.
Mix well and allow cooling before use.
Label name of reagent, date of Preparation, signature of staff preparing.

Sample Required: Sputum samples/specimens

Sample Collection.

- Sputum is collected into a wide mouthed container taking care not to smear the container and also not to create aerosols.
- The patient is required to give a spot sample; collected on the initial visit and a morning sample collected the following day.
- Sample collection should be done in an open area, preferably in the sun since the UV rays destroy mycobacterium.
- Instruct the patient on how to produce sputum by deep inhalation thrice or twice and then coughing deeply to avoid saliva specimens.

Procedure

Smearing

- Label the slide with the serial number and collection number of the sputum specimen.
- Open the sputum container carefully to avoid/minimize aerosol production.
- Using the broken applicator stick, select the most purulent particles of the sputum using the jagged edge.
- Make a smear 2cmx1cm and allow to air – dry.

- Discard used sticks in a container containing 5% aqueous phenol solution or 5% sodium hypochlorite solution, then autoclave or incinerate.

Fixing

- Heats fix the dried smear by holding it with forceps and passing it side up through the flame 5 times or for about 4 seconds.
- Do not heat fix moist smears and do not over heat.

Staining

- Place the fixed smears on the staining rack smeared side up. Place a 1cm gap between the sliders to avoid contact with each other.
- Cover the slides individually with filtered 1% carbol fuchsin.
- Heat the slides from underneath until vapour starts to rise. Do not boil.
- Keep the hot carbol fuchsin at the slides for 7 minutes.
- Rinse the slides gently with water to remove the stain
- Tilt to remove excess water.

Decolourising

- Cover the slides with 25% sulphuric acid solution for 3 minutes
- Repeat if need be when the red colour is persistent.
- Gently wash away the sulphuric acid with water and tilt the slide to remove excess water.

Counterstaining

- Cover the slides individually with 0.1% methylene blue counter staining solution and allow standing for 1 minute.
- Rinse the slides individually with water and tilt the slide to remove excess water.
- Allow to air dry the rack.

REPORTING AND RESULT INTERPRETATION

- View AFB slide under oil immersion x100 objective
  No. of bacilli found relates to: degree of infectivity of patient and severity of disease.
- Viewing is done systematically moving from left to right which is equivalent to 100 fields (2 horizontal and 3 vertical lines)

AFB Counts seen	what to report
- No AFB in 100 fields	negative (o)
- 1-9AFB in 100 fields	actual number of 1,9,4
- 10-99AFB in 100 fields	1+
- 1-9AFB in one field in at least 50 fields	2+
- >10AFB in one field in about 20 fields	3+

Quality control procedure

- Label all AFB stains with date of preparation, concentration and initials of staff preparing the stain.
- Counter – check all new stains against confirmed positive and negative counter sliders before use.

- Use known positive (+) and negative control slides when staining patient samples.
- Remove oil immersion using xylene or by putting slides upside down on a tissue or gauze.
- When storing slides do not differentiate positive and negative smears.
- Retain all slides for external quality assurance.

Safety precautions

- Avoid creating /production of aerosols when making smears. This is done by preventing vigorous opening and handling of the sputum specimen, heating moist smears.
- Avoid standing in front of the patient during sample collection.
- If available, process the specimen in a safety hood.
- Avoid overheating the smears during staining.
- Reject contaminated/soiled sputum containers to avoid infection.

Trouble shooting/sources of error

- Use new slides to avoid false positive results.
- Clean the microscope lens using the lens tissue.
- Ensure new batches of stains are quality controlled using known slides; positive and negative.
- Over decolouring with 25% sulphuric acid.

References

- Collins CH, Grange JM, Yates MD
  Tuberculosis bacteriology 2nd edition 1997
- International union against TB and Lung disease (IUATLD)
  Technical guide – sputum examination for TB by direct microscopy in low income countries.
- Monica Cheesebrough volume 1 and 2.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE
CRAG.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE

The LATEX-CRYPTOCOCCUS ANTIGEN TEST is based upon the principle that anti Cryptococcus anti-body coated latex particles will agglutinate with specimen containing capsular polysaccharide antigen (4, 6). previously, the detection of this antigen in serum was hampered by the presence of rheumatoid factor (3,15) pre-treatment of serum specimens with Pronase (REF DEOO10) reduces non-specific interference and enhances the detection of capsular polysaccharide antigens of c.neoformans (17)due to rheumatoid factor (34) and immune complexes (33).

Clinical significance

- Cryptococcosis.
- Meningencephalitis.
- Cryptococcal pneumonia.

Equipment

- Lab rotation.
- Water bath.

Equipment maintenance schedule

3 months times

Reagent preparation

a) Pronase (REFDE0010) 1.75ml reconstituted Pronase should be aliquoted into test tubes in 0.05ml (50ul) amounts, sealed and frozen immediately at -20°c or colder. Do not use siliconized tubes.
b) Specimen diliuent (REFGB0020) dilute 1:10.
c) Latex solutions must appear as homogeneous suspensions.
d) When using the negative control (REF N 80110) for the first time inactivate at 56°c for 30 minutes.
e) The ring slides (REF SC0020) should be discarded after each use.

Sample required

- Serum.
- CSF (cerebral spinal fluid).

Sample collection

- CSF: - Collected asceptically by lumber puncture technique by a qualified Doctor.
- Serum: - Collect blood in a tube containing clot activator and allow it to clot; spin for 2 minutes at a 1000RPMs and collect the serum.

Procedure.

1. Add 25ul of Cryptococcus antigen positive control, negative control and each heat treated C.S.F and/or Pronase treated serum specimen onto separate rings of the ring slide.
2. Use a new pipette tip for each reagent and specimen.
3. Add 25ul of cryptococcal latex to each ring.
4. Using separate applicator sticks, thoroughly mix the contents of each ring.
5. Rotate at 100 rpm at 5 minutes at room temperature.
6. Read reactions immediately.

READING THE TEST.

Read the reactions immediately over a dark background and rate them on a scale from negative to 4+. Do not magnify. For comparison, the Cryptococcus Antigen Positive control should give a 2+ or greater reaction and the Negative should be less than 1+.

Negative (-); *a homogenous suspension of particles with no visible clumping.*
One plus (1+); *fine granulation against a milky background.*
Two plus (2+); *small but fine clumps against a slightly cloudy background.*
Three plus (3+); *large and small clumps against a clear background.*
Four plus (4+); *large clumps against a very clear background.*

Quality Control

Latex Control

Periodically, the sensitivity of the cryptococcal latex reagent may be tested by titering the cryptococcal antigen positive control. The cryptococcal antigen positive control should titre 1; 4(+/-) 1 dilution if the sensitivity of the cryptococcal latex reagent is satisfactory

Pronase Control

A*t least once a month, a frozen aliquot of Pronase should be tested for proteolytic activity by substituting a 300ul aliquot of Pronase control for specimen in steps 4 – 6in serum specimen preparation. Both the Pronase treated sample of Pronase control and an untreated sample of Pronase control should be tested simultaneously using the screening procedure above*
The untreated Pronase control must be 2+ or greater and the Pronase treated Pronase control must be less than 1+, then the proteolytic activity of Pronase has diminished and a new vial of Pronase should be rehydrated, aliquoted, frozen and tested.

CONTROL REACTIONS

A Positive reaction with the Negative control may indicate possible contamination or freezing of the cryptococcal latex, which could produce false positive results in the patient specimens. The Pronase control detects the presence of rabbit globulin on the latex particles. Failure of the Pronase control to give a positive reaction indicates that one of the reagents is unsatisfactory.

LIMITATIONS OF THE PROCEDURE

A negative result does not exclude the possibility of cryptococcal infection, particulary when a single patient specimen has been tested and the patient has symptoms consistent with Cryptococcosis.

CAUSES OF FALSE NEGATIVE REACTIONS

1. *Low titres.*
2. *Early infection.*
3. *Presence of immune complexes.*
4. *Prozone effect of high titres.*
5. *Poorly encapsulated strains with low production of polysaccharide*

CAUSES OF FALSE POSITIVE REACTIONS

1. Presence of rheumatoid factor.
2. Agar syneresis fluid.
3. Sera with > 200mg iron/dl.
4. Improper cleaning of the ring slide.
5. Non specific reactivity in H.I.V infected patients.
6. Increased titres.
7. Increased sensitivity in serum specimens.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE (SOP) FOR URIC ACID.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST

It's based on end point analysis (uricase) and peroxide).
Uric acid + O2 + 2H2O uricase Allantoin + CO2 + H2O2.
H2O2 + DCHBS + PAP _peroxidase quinoneimine+HCL+4H20.
The increase in absorbance generated by the red dye is proportioned to the uric acid concentration in the sample.

CLINICAL SIGNIFICANCE

Used in the diagnosis and Rx of gout and impaired renal functions.

EQUIPMENT

- Automated Humastar 180 and semi-automated Humalyser 2000.

EQUIPMENT MAINTENANC SCHEDULE

- Routine service schedule after 6 months.
- Daily calibration.
- Daily QC run.

REAGENTS AND MATERIALS

- Ready to use reagent are supplied.
- Pipette, pipette tips, sample caps.

REAGENT PREPARATIONS AND STABILITY

- Reagent and standard are ready for use.
- Reagents are stable, even after opening upto expiration date when stored @2 - 8°C.
- If stored at 15 - 25°C, protected from light, reagent is stable for 2weeks.
- Contamination of the reagents must be absolutely avoided.

SAMPLE REQUIRED – Serum, heparinised Plasma or urine.

SAMPLE COLLECTION

- Select the best vein from the arm of the patient.
- Tie it with toniquet.
- Sterilize the site using spirit swab.
- Withdraw about 3mls of blood and put in a heparin tube or tube with clot activator.
- Spin it 1000rpm for 2 minutes

– Plasma is obtained and it’s usually used for analysis.

PROCEDURES

- Put about 500 ul of the plasma into the sample cap.
- Place the reagent and sample in designated slots in the Humastar 1800.
- Follow the machine’s software commands.

REPORTING AND INTERPRETATION OF RESULTS

The activity is expressed in mg/dl or umol/l.

REFERENCE RANGE/NORMAL VALUES

- Male 200 – 420 Umol/l
- Female < 140 – 340 Umol/l

NB: They vary with age, diet, gender, and geographical area.

Internal Quality Control

Running the ready manufactured normal and high controls.

Trouble shooting/source of error

QC and calibration not passing.

Safety Precautions

- Avoid reagent contact with skin, eyes and mucous membrane.
- Wear suitable protective clothing’s plus gloves.
- When emptied into the drain, flash with lots of water to neutralise the chemical effect of the reagent.
- Do not swallow reagent.

Performance characteristics.

- Dilute samples with high concentration 1+1 with physiological saline (0.9%).multiply the results by 2.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: ....*

Approved by: *Signature: …………………….*

****

STANDARD OPERATING PROCEDURE (SOP)
FOR GOT (ASAT) (GLUTAMIC OXALACETIC TRANSAMINASE)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST

It's based on IFCC modified method without pyridoxalphosphate activation
L-Aspartate + 2-Oxoglutarate GOT L- glutamate + oxaloacetate.
Oxalacetate + NADH + H Malate dehydrogenase L- Malate + NAD+
The rate of decrease in absorbance due to oxidation of NADH to NAD+ is directly proportional to sample activity of ASAT.

CLINICAL SIGNIFICANCE

Its measurements are used in the diagnosis and treatment of certain types of liver and heart diseases.

EQUIPMENT

- Automated Humastar 180 and semi automated Humalyser 2000.

EQUIPMENT MAINTENANC SCHEDULE

- Routine service schedule after 6 months.
- Daily calibration.
- Daily QC run.

REAGENTS AND MATERIALS

- Ready to use reagent are supplied.
- Pipette, pipette tips, sample caps.

REAGENT PREPARATIONS AND STABILITY

- Pipette 2ml of substrate into one bottle of Buffer, mix thoroughly and the reagent is ready for use.
- The working reagent is stable for 4weeks @ 2 - 8° C and 5 days at 15 - 25° C.

SAMPLE REQUIRED – Serum or heparinised Plasma.

SAMPLE COLLECTION

- Select the best vein from arm of the patient.
- Tie it with toniquet.
- Sterilize the site using spirit swab.
- Withdraw about 3mls of blood and put in a heparin tube.
- Spin it 1000rpm for 2 minutes.
- Plasma is obtained and it's usually used for analysis.

PROCEDURES

- Put about 500 ul of the plasma into the sample cap.

- Place the sample and reagent in designated slots in the machine.
- Follow the machine's software commands.

REPORTING AND INTERPRETATION OF RESULTS

The activity is expressed in international units/liter (u/l) which is defined as enzyme activity that converts 1 Umol of substrate in 1 minute.

REFERENCE RANGE/NORMAL VALUES

- Male < 37 U/L @ 37 °c.
- Female < 31 U/L @ 37 °c.

NB: They vary with age, diet, gender, and geographical area.

Internal Quality Control

Running the already manufactured normal and high pooled plasma.

Trouble shooting/source of error.

Failing to calibrate the machine and running QC.

Safety Precautions

- Avoid reagent contact with skin and eyes
- Wear suitable protective clothing's plus gloves
- Do not empty the reagent into drains.

Performance Characteristics.

- In sera with very high activities, the initial absorbance may be very low as most of the NADH may have been consumed before the first reading. In this case rerun the sample after dilution.
- If absorbances change per minute or the activity exceed dilute 0.1ml of the sample with 0.9ml physiological saline (0.9%) and repeat the assay using this dilution. Multiply the result by 10.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

.

STANDARD OPERATING PROCEDURE (SOP) FOR HEPATITIS C VIRUS ANTIBODY.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST

During testing the serum/plasma specimen reacts with the protein A coated particles. The mixture migrates upwards on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a coloured line which indicate a +ve results and it absence indicate –ve results.

CLINICAL SIGNIFICANCE

For qualitative detection of antibody to Hepatitis c Virus.

EQUIPMENT

- Timer, centrifuge.

EQUIPMENT MAINTENANCE SCHEDULE

- Routine service schedule after 6 months.
- Oiling of movable centrifuge parts.
- Daily QC.

REAGENTS AND MATERIALS

- Test strips, EDTA Tube, Heparin tube or plain tube, sample diluent, plastic dropper.

REAGENT PREPARATIONS

- Ready to use strips is supplied.

SAMPLE REQUIRED – Blood, Serum or Plasma.

SAMPLE COLLECTION

- Select the best vein from of the patient.
- Tie it with toniquet.
- Sterilize the site using spirit swab.
- Withdraw about 3mls of blood.
- Specimen is ready to be processed.

PROCEDURES.

- Bring the pouch to room temperature before opening it.
- Spin the blood to obtain plasma or serum
- Transfer about 0.5mls of plasma/serum to a clean test tube.
- Remove the test strip from the sealed pouch and use it as soon as possible.
- With arrows pointing downwards drop the test strip into the test tube containing plasma/serum for at least 10-15 seconds.

- Remove the test timer and wait for the red line to appear for 15 minutes.
- Read the results.

NB; DO NOT INTERPRATE RESULTS AFTER 20 MIN.

REPORTING AND INTERPRETATION OF RESULTS

Positive – Two distinct red lines appears one in area © and another in area (T) on the Test strip.

Negative – One red line appears in the control region ©.

Invalid – if there is no purplish red control band in the control region regardless of test result, the test is considered invalid. Repeat the test using a new test device.

Internal Quality Control

Use of normal saline on the test strip.

Trouble shooting/source of error

Invalid results: where lines fails to appear due to insufficient specimen.

Safety Precautions.

- Wear protective gloves.
- Discard the used test strips as high risk specimen.

Warnings and precautions

- All positive results must be confirmed by an alternative method.
- Treat all specimens as though potentially infectious. Wear gloves and protective clothing when handling specimens.
- Do not use kit material beyond their expiration dates.
- Do not interchange reagents from different lot of kit.

Limitations

1. Only samples that are clear and with good fluidity can be used in this test.
2. Fresh samples are best but refrigerated samples can be used.
3. Do not agitate the sample. Insert a pipette just below the surface of the sample to collect the specimen.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE (SOP FOR KOH (POTASSIUM HYDROXIDE).

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST

KOH digest the keratin surrounding the fungi so that the hyphae and conidia (spores) can be seen by the use of microscope.

CLINICAL SIGNIFICANCE

Help to identify fungal infections.

EQUIPMENT

- Microscope, microscope glass slide, cover slips, incubator timer.

EQUIPMENT MAINTENANC SCHEDULE

- Routine service schedule after3 months.
- Regular cleaning.

REAGENTS AND MATERIALS

- Potassium Hydroxide (20% w/v).
- Scalpel blade.
- Glass slide and cover slips.
- Tissue or gauze.

REAGENT PREPARATIONS

Potassium Hydroxide (20% w/v)

- Potassium Hydroxide (KOH) – 10g.
- Distilled water – 50ml.
- Weigh the KOH powder and transfer to a screw-cup bottle.
- Add water and mix until the chemical is completely dissolved.

SAMPLE REQUIRED – Skin scales, nails, hair.
SAMPLE COLLECTION

- Cleanse the affected area with 70% ethanol
- Collect skin scales, crusts, pieces of nails or hairs on a clean piece of paper.

Skin scales: collect by scraping the surface of the margin of the region using a sterile scalpel.

Crusts: collect by removing part of the crust nearest to healthy skin using sterile Scissors.

Hairs: collect by removing dull broken hairs from the margins of the lesion using a Tweezers.

Nail pieces: collect by taking snipping of the infected part of the nail using sterile scissors.

PROCEDURES.

- Place a drop of potassium hydroxide solution on a slide.
- Transfer the specimen (small pieces) to the drop of KOH and cover with a cover glass.
- Place the slide in a Petri-dish together with a damp piece of tissue or gauze.
- Incubate the preparation in an incubator at 37°c for 20-30 minutes.
- Remove and examine microscopically using x10 and x40 objectives.

REPORTING AND INTERPRETATION OF RESULTS

Look for branching septae with angular or spherical arthroconidia (arthrospores) usually in chain.

.

Internal Quality Control

Done after preparation of a new reagent using a known +ve specimen.

Trouble shooting/source of error

- Short incubation time.
- Expired reagent.

Safety Precautions

- KOH reagent is corrosive therefore should be handled with care.
- Wear protective gloves throughout the procedure.

Reference

- Laboratory practice in tropical countries by Monicah Cheesebrough.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE (SOP) FOR HEPATITIS B SURFACE ANTIGEN HBsAg.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST.

During testing the serum/plasma specimen reacts with the particles coated with anti-HBsAg antibody. The mixture migrates upwards on the membrane chromatographically by capillary action to meet with anti-HBsAg antibodies on the membrane and generate a colored line which indicate a +ve results and the absence indicate a –ve result.

CLINICAL SIGNIFICANCE.

For qualitative detection of Hepatitis B surface antigen.

EQUIPMENT.

- Timer, centrifuge.

REAGENTS AND MATERIALS.

- Test strips, plain tube, EDTA tube.

REAGENT PREPARATIONS.

- Ready to use strip is supplied.

SAMPLE REQUIRED – Blood, Plasma, and Serum.

SAMPLE COLLECTION.

- Select the best vein from the patient hand.
- Clean the area with the spirit swab.
- Withdraw about 3mls of blood.
- Specimen is ready to be processed.

PROCEDURES

- Bring the pouch to room temperature before opening it.

- Spin the blood to obtain plasma or serum.
- Transfer about 0.5mls of plasma/serum to a clean test tube.
- Remove the test strip from the sealed pouch and use it as soon as possible.
- With arrows pointing downwards deep the test strips into the test tube containing plasma/serum for at least 10-15 seconds.
- Remove the test strip and place on a non-absorbent flat surface, start the timer and wait for the red lines to appear or 15 minutes.
- Read the results.

REPORTING AND INTERPRETATION OF RESULTS

Positive – Two distinct red lines appear. One in area © and another in area (T) on the Test strip.

Negative – one red line appears in the control region ©.

Internal Quality Control

Use of normal saline in the test strip.

Trouble shooting/source of error

- Invalid results: due to insufficient sample.

Safety Precautions

- Discard used test strips as high risk waste.
- Wear protective gloves throughout the procedure.

Reference

- Medical laboratory practices part 2 by Monicah Cheesebrough.
- Package insert.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: ....*

Approved by: *Signature: ………………………..*

****

STANDARD OPERATING PROCEDURE (SOP)
FOR DIRECT COOMB TEST.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2018

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST

When sensitized baby's blood (washed cells) is mixed with AHG and incubated for 45 minutes it forms agglutination which can be seen both microscopically and macroscopically.

PURPOSE OF TEST

It checks sensitization in vivo (inside the body).

EQUIPMENT

- Incubator/water bath, Microscope, Timer, Centrifuge.

EQUIPMENT MAINTENANCE SCHEDULE

- Schedule after3 months.
- Regular cleaning.

REAGENTS AND MATERIALS

- AHG.
- Glass slide and test tubes.
- Normal saline.

REAGENT PREPARATIONS

- Normal Saline – mix 8.5gms of Nacl in 1000 liter of distilled water.
- AHG ready to use.

SAMPLE REQUIRED – whole blood.

SAMPLE COLLECTION.

- Locate the best vein from the baby.
- Sterilize the site using spirit swab.
- Withdraw about 3mls of blood and transfer to an ETDA tube.
- Specimen is ready for analysis.

PROCEDURES

- Take a tube and label DC.

- Add 2 volumes of baby's cells.
- Wash the cells 3 times in large volume of normal saline.
- Discard the supernatant and add 1 volume of AHG.
- Centrifuge at 100rpm for 1 minute.
- Examine both macro and microscopically.

REPORTING AND INTERPRETATION OF RESULTS.

Agglutination – positive.
No agglutination – negative.

Trouble shooting/source of error

- Using expired reagent.

Safety Precautions.

- Wear protective gloves throughout the procedure.

Reference.

- Lab handouts.
- District laboratory practices part 2.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE (SOP) FOR INDIRECT COOMB TEST.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST.

When sensitized blood serum is mixed with O positive pooled cells under temperature of 37°c for 1 hour it forms agglutination.

PURPOSE.

To check sensitization invitro (outside the body).

EQUIPMENT.

- Microscope, incubator, Timer, centrifuge.

EQUIPMENT MAINTENANCE SCHEDULE.

- Routine service schedule after 6 months.
- Regular cleaning.

REAGENTS AND MATERIALS.

- Test tubes, glass slide.
- AHG.
- Normal saline.
- O+ve pooled cells.

REAGENT PREPARATIONS.

Normal saline.

- Mix 8.5gms of Nacl in 1000 little of distilled water.
- AHG ready to use.

SAMPLE REQUIRED – whole blood.

SAMPLE COLLECTION.

- Locate the best vein from the arm of the patient
- Sterilize the site using spirit swab.
- Withdraw about 3mls of blood and transfer to U tube containing clot activator.
- Spin at 1000rpm for 3 minutes.
- Separate serum from red cells and it's ready for analysis.

PROCEDURES

- Prepare O+ve pooled cells by mixing different blood from different donors.
- Do cell washing 3 times.
- Label anti human globulin tube (AHG).
- Add 1 volume of O+ve 4% cell suspension and 1 volume of patient's serum.
- Incubate at 37°c for about 1-2 hours.
- After incubation wash the mixture 3 times with normal saline. Discard completely the saline after the last wash.
- Mix the cells and add 1 volume of AHG mix and centrifuge at 1000rpm for 1 minute.
- Examine both macroscopically for the presence or absence of agglutination.

REPORTING AND INTERPRETATION OF RESULTS.

Agglutination – positive.
No agglutination – negative.

Internal Quality Control.

Carrying out the procedure from Unknown positive and negative known samples.

Trouble shooting/source of error.

- Using expired reagent.

Safety Precautions.

- Wear protective gloves throughout the procedure.

Reference

- District laboratory practice part 2.
- Laboratory handouts.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: ....*

Approved by: *Signature: ……………………..*

****

STANDARD OPERATING PROCEDURE.
RHEUMATOID FACTORS.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of test

RF slide test for detection of rheumatoid factor is based on the principle of agglutination. The test specimen (serum) is mixed with RF latex reagent and allowed to react. If RF is present then a visible agglutination is observed. If RF is absent then no agglutination is observed.

Clinical significance/purpose of the test.

►Helps in detecting rheumatoid arthritis.

Reagent and Materials.

- Glass slides with six reaction circles.
- Lab Rotator.
- Mixing Stick.
- RF latex reagent.
- Blood collection needles.
- Tube holders.
- Plain tubes.
- Tourniquet.
- High intensity direct light source.
- Isotonic saline.
- Positive control.
- Negative control.

Equipment.

►Lab Rotator.
►Stop watch.

Sample Required: Serum.

Test Procedure.

Bring all reagents and samples to room temperature before testing.

Qualitative Method.

- Pipette one drop of serum onto a glass slide using a disposable pipette provided with the kit.
- Add one drop of RF latex reagent to the test specimen on the slide. Do not let the dropper tip touch the liquid on the slide.
- Using a mixing stick, mix the serum and RF latex reagent uniformly over the entire circle.

- Immediately start a stop watch. Rock the slide gently, back and forth, observing for agglutination macroscopically at two minutes.

Semi quantitative Methods.

- Using isotonic saline prepare serial dilutions of the serum sample e.g. 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 etc.
- Pipette each diluting of the serum sample on separation reaction circle.
- Add one drop of RF latex reagent to each drop of the diluted serum samples on the slide.
- Using a mixing stick, mix the sample and latex reagent uniformly over the entire circle.
- Immediately start a stop watch. Rock the slide gently, back and forth observing for agglutination macroscopically after two minutes.

Interpretation of the results

Qualitative Methods.

►Agglutination is a positive test result and indicates the presence of rheumatoid factors in the test specimens.

No agglutination is a negative test result and indicates the absence of rheumatoid factor in the test specimens.

Semi Quantitative Method.

►Agglutination in the highest serum dilution corresponds to the approximate amount of rheumatoid factors in IU/ML.

To calculate rheumatoid factors in IU/ML, use the formula:

RF (IU/ML) =10Xd

Where D=highest dilution of serum showing agglutination.

Quality Control.

►Only a clean and dry glass slide must be used. Clean the slide with distilled water and wipe dry.

Sources of Errors.

►Use of dirty slides.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
CSF PROFILE

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of test.

- Ziehl – Neelsen smear when tuberculosis is suspected.

This is done when tuberculosis meningitis is clinically suspected or the CSF contains lymphocytes and the glucose concentration is low and protein levels raised.
However AFB is difficult to detect in CSF.
The stated down procedures increases the chances of finding of bacteria.

Procedure:

- Centrifuge the CSF at high speed for 20-30 minutes. Remove the supernatant fluid and mix the sediment. Transfer several drops of the sediment to a slide, allowing each drop to dry before adding the next.
- Fix the dry preparation with methanol and stain following the ZN technique steps.
- Examine the smear first with x40 objective to see the distribution of materials and then x100 objective to detect AFB. Examine the entire preparation.

Indian ink preparation when cryptococcal meningitis is suspected
When cryptococcal meningitis is clinically suspected, e.g. patient with HIV disease, or when yeast cells are detected when performing a cell count or examining a gram smear, examine an Indian ink preparation or a wet preparation by dark field microscopy for encapsulated yeasts.

Procedure:

- Centrifuge the CSF for 5-10 minutes. Remove the supernatants fluid and mix the sediment.
- Transfer a drop of the sediment to a slide, cover with a cover glass and examine by dark field microscopy or add a drop of Indian ink, mix and cover with a cover glass.
- Examine the preparation using x40 objective.
- Look for oval or round cells, some showing budding, irregular in size measuring 2-10ml in diameter and surrounded by a large unstained capsule.

Gram staining:

This is required when the CSF contains pus cells (neutrophils) it should be the first investigation to be done and reported when the CSF appears purulent, or turbid. It is also important when a CSF is unsuitable for cell count or biochemical testing e.g. when heavily blood stained or contains clots.

Procedure:

- Centrifuge approximately for 5-10 minutes.
- Transfer the supernatant fluid to another tube (incase glucose and protein estimation is needed).
- Mix the sediment, transfer a small amount into a slide and make a smear.
- Alcohol fix the preparation and follow the gram staining procedure steps.

Results:

- Examine the smear x40 objective and x100 objective for pus cells and bacteria.

Reporting.

Pus cell – report as moderate, many or few.
Bacteria – gram –ve intracellular diplococci which could be N.meningitidis

- Gram +ve diplococci could be S.pneumoniae.
- Gram –ve rods possibly H. influenza, E.coli.
- Yeast cells.

APPOVAL.
The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
ERYTHROCYTE SEDIMENTATION RATE (WESTERGREN TECHNIQUE).

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Clinical significance of the test:
ESR is a non specific test which is raised in a wide range of infections, malignancies, degenerative conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulin's and c-reactive proteins. Other factors affecting ESR include: pregnancy, haemoglobinopatheis, haemoconcentration and treatment with anti-inflammatory drugs.

Principles of the test
When citrated blood is allowed to stand in a vertically positioned westergren tube and left undisturbed, red cells aggregate, stack together to form rouleaux and sediment through the plasma. The ESR is the rate at which this sedimentation occurs in one hour. Sedimentation is increased when the ratio of red cells to plasma is altered e.g. in anaemia and is reduced when the red cells are abnormally shaped e.g. sickle cells. High temperatures >25°c increase sedimentation.

Equipment.
- Westergren tubes (disposable).
- Westergren stand/plastic stand.
- Timer (1hr).
- Sample tubes with anticoagulant (disposable).

Reagent.
Sodium citrate 3.2%w/v anticoagulant.

Sample.
- Citrated blood.

Procedure.
- Put blood into the sample tube with the anticoagulant.
- Place the tube into the holder and firmly press the westergren tube into the tube ensuring no air bubbles are formed. Ensure the blood level gets to the 0 mark.
- Rest the tube in the rack and start the 1hr timer.

- After exactly 1 hour, read the level at which the plasma meets the red blood cells in min.
- After reading, dispose the blood safely.

Interpretation of ESR tests Results:

Reference range:

- Men – up to 10mm/hr.
- Women - up to 15mm/hr.
- Elderly – up to 20mm/hr.

Safety precautions.

- Always wear protective clothing when performing this procedure.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
CREATININE ESTIMATION

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.18 *Revision Date: 01.10.18*
Next Revision Date: 02.10.19

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Clinical significance
Creatinine measurements are used in the diagnosis and treatment of renal disease and in monitoring renal dialysis.

Principles of the test
- It's a fixed analysis, colorimetric methodology based on the reaction of creatinine with picric acid under alkaline conditions.

Creatinine + picrate → red complex
The increase in absorbance due to red complex is proportional to the creatinine concentration in the sample. Absorbance measurements are taken as 510um.

Equipment
- Chemistry analyser
- centrifuge

Reagent -CREA R1 sodium hydroxide
-CREA R2 picric acid

Reagent preparation
Reagents are ready to use
After daily use, cap the bottle and store at 15-25°c

Specimen
- serum

Specimen storage
Specimen may be stored for 24 hours at 2-8°c, freeze for longer storage

Procedure
Follow the software commands to run the test.

Quality control
Normal and abnormal controls level 1 and level 2 are used to complete quality control program.

Reference interval.
Serum:
- Male 0.7 to 1.3mg/dl (62-115 umol/l)
- Female 0.6 to 1.1mg/dl (53-97umol/l)

NB: Reference ranges may vary with age, diet, gender and geographical area.

Safety precautions

- Always wear protective clothing when running the procedure.
- Always ensure the requirements of the machine are met before running the test i.e. calibration of machine, cleaning and blanking.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE HAEMOGLOBIN LEVEL ESTIMATION USING DIASPECT TM

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principles of the test.

Undiluted whole blood is drawn into chemically coated single use microcuvettes. Sodium deoxycholate haemolyses the erythrocytes and haemoglobin is released. Sodium nitrate converts haemoglobin to met haemoglobin which together with sodium oxide gives azidemethaemoglobin. The absorbance is measured at two wavelengths (570um and 880um) in order to compensate for turbidity in the sample.

Reagent.

- 40% w/w sodium deoxycholate.
- 18% w/w sodium azide.
- 20% w/w sodium nitrite.
- 22% w/w reactive ingredients.

Hemocue 201+ Hb microcuvettes.
Spirit swabs.
Pickers.
Cotton gauzes.

Sample

- Capillary, venous or arterial blood may be used
  Anticoagulants in solid form e.g. EDTA or Heparin/fluoride are recommended to avoid a diluting effect.
- If the blood has been refrigerated, it must be allowed to reach room temperature before analysis.

Procedure.

- After start up, the curette holder should be in its loading position, display showing the three flashing dashes and Hemocue symbol.
- If collecting blood from fingertip, ensure the patients hand is warm and relaxed. Use only the middle or ring finger for sampling. Clean the finger with disinfectant and allow to dry, prick using a lancet, wipe off the first 2 drops of blood. Apply slight pressure till another drop appears.
- When large enough, fill the micro curette in one continuous process. Do not refill.
- Wipe off the excess blood on the outside of the curette making sure no blood is drawn out of the micro curette. Ensure there are no air bubbles.
- Put the cuvette holder to its measuring position.
- After 15-60 seconds the haemoglobin value is displayed.

- When measuring various blood, mix the blood well before performing the measurement. Place a drop of blood into a hydrophobic surface e.g. a plastic film.

Reference ranges.

- Adult males 13.0-17.0g/dl
- Adult female 12.0 – 15.0g/dl
- Infants (after neonatal period) 11-14g/dl.

Quality Control.

- Hemocue Hb 201+ analyser has an internal electronic 'self-test'. Every time the analyser is turned on it will automatically verify the performance of the optic unit of the analyser.

Trouble shooting.

- Hemocue Hb 201+ analyser has a trouble shooting guide (ref pg 30-32).

Safety precautions.

- Always wear protective gloves when performing the whole procedure.
- Always store the curettes at room temperature and in a properly closed container.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
SERUM AMYLASE.

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle
5C NPG3 α amylase 3CNP +2CNPG2+3G3 +2.
The rate of increase of absorbance is due to the production of 2-chloro-4-nitraphenol (CNP) and is directly proportional to the amylase activity in the sample.

Clinical significance
1L test amylase is intended for quantitative in vitro diagnostic determination of amylase in human serum, plasma or urine using Humastar 180 chemistry system.

Equipment
- *Centrifuge,*
- *chemistry analyzer*

Equipment Maintenance
- Centrifuge lubrication done on movable part after every 3 months.
- General services after 6 months.
- 1 lab 300 plus chemistry analyzer: general services done after 6 months
- Wiping the external parts with decontaminator daily e.g. jik.

Reagent:
Reactive reagents containing
- 2 – chloro -4- Nitrophenyl – oc maltotriside
- Sodium chloride
- Calcium acetate
- MES buffer PH 6.0
- Potassium thiocynate

Material:
Posterior pipette, micro pipette, reaction curette, lithium heparin containing tubes, sample cups, pipette filler and test tube rack.
Reagent preparation: The amylase R2 reagent is supplied ready to use uncap and place into reagent tray.
Sample required
Serum plasma

Serum collected into red tube (clot activator).
Plasma collected into green tube (lithium heparin).

Sample Collection

- Collect various blood by clean and sterile venipuncture
- Serum allow blood to clot properly and centrifuge
- Plasma collect blood into tube containing lithium heparin anticoagulant. Centrifuge at 3000xg for 3-4 minutes.

Procedure

- Feed the computer with patient details
- Transfer serum/plasma into the sample cup
- Place it into sample tray
- Replace the sample tray
- Start on to analysis the patient sample.

REPORTING AND RESULT INTERPRETATION

- The results are displayed on the computer screen with two different colours that is black and red each with different meaning.
- Black colour indicates the results are in normal range
- Red colour indicates the results are either below or above normal range.

Reference range: 27 -102 u/l or 450 – 1700nkat/l

Internal Quality control

- Normal and abnormal serum control commercially available are run daily before analysing patient sample.
- Patient sample is only run when the normal serum control are within given normal range or abnormal serum control are within given normal range.

Trouble shooting/sources of error

- Re – calibrate the test
- Re – washing reaction curette
- Changing reaction curette with absorbance below or above given range by manufacturer.

Safety precautions

- Always wear protective cloths
- Always wear disposable latex gloves when handling patient sample.
- Always empty the waste in sink while tap water is running.
- Clinical chemistry book by Sitati
- Insert user manual from kits

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T Signature: .....
Prepared by: Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE
ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST (APTT)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle

The APTT test measures the clotting time of test plasma after adding of APTT reagent then allowing an "activation timer" followed by the addition of Cacl2. Deficiencies of approximately 40% and lower of factors V111, 1X, X1 and X11 will result in prolonged APTT, Heparin, in the presence of adequate amounts of AT -111 will also result in prolonged APTT.

Clinical significance

- The test helps to screen the intrinsic clotting system. It will detect the inhibition or deficiency of one or more of the following factors: V111, IX, X, XI, XII and fibrinogen.
- It is also used to monitor patients being treated with heparin.

Equipment

- *Cacl2*
- *Kaolin/platelet substitute mixture*

Materials

- Yellow tips, micro pipette, reaction curette

Reagent Preparation

- Commercial available (ready for use)

Sample required: plasma only collected into the tube with sodium citrate.

Sample Collection

- Obtain venous blood by clean venipuncture
- Immediately combine 9 part blood with 1 part anticoagulant mixing well by inverting the tube against the stopper.
- Centrifuge the sample at 3000xg for 10 min.
- Test plasma sample within 4 hours.

Procedure

- Pipette 25ul plasma into curette
- Add 25ul APTT to plasma
- Incubate exactly for 5 minutes
- Transfer curette to measuring position.
- Activate optic (press key optic)
- Add 25 ul prewarmed calcium chloride (measurement start automatically)
- The instrument will read to a maximum of 300sec.
- The result is displayed in seconds and ratio.

Reporting

- Results are automatically displayed on screen and reported to the nearest 0.1 seconds. Above the normal and one abnormal control be run at least once in a day.

Trouble shooting

- Washing the Huma clot with methylated spirit
- Re- calibration of the machine

Safety precautions

- Always wear protective cloth when handling blood samples
- Always wear disposable latex gloves

References

- Baker
- Monicah Cheesebrough part 11
- Insert manual

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE
PROTHROMBIN TIME (P.T)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test
The one stage PT measures the clotting time of plasma after adding a source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated factor xa with the consequent formation of thrombin and ultimately an insoluble fibrin clot.

Clinical significance
Thromboplastin – 51 (PT – 51) is highly sensitive reagent (ISI 1.0 -1.3) that is used to perform the one stage prothrombin time (PT). Prolongation of the PT indicates either acquired or congenital disorders that affect coagulation factor 1, 11, V, VII and X. PT is widely accepted as the means to monitor patient with oral anticoagulant therapy.

Equipment
- *Centrifuge, humanclot*

Equipment maintenance
Twice in a year
Wiping the machine properly using spirited swabs.

Reagent: Thromboplastin reagent
Materials: Micro pipette, reaction curette, tips

Reagent preparation: Reconstitute with 2ml (REF) 31002) of distilled water. Agitate gently and let the vial stand undisturbed for 15 minutes at room temperature.

Sample Required: Plasma only collected into the tube with anticoagulant sodium citrate.

Sample Collection
- Collect various blood by clean venipucture.
- Immediately mix 9 parts blood with 1 part anticoagulant.
- Avoid foaming the specimen

Procedure
- Pipette into pre-warmed test tube.
- Incubate 3-5 minutes at 37°c.
- Add pre-warmed reagent
- Start timer with addition of reagent.

- Machine gives automatic result when clot formed.

REPORTING AND RESULT INTERPRETATION

- Results are automatically displayed on screen and reported to the nearest 0.1 seconds.
- ANR is also displayed on screen automatically. Above the normal time reported as abnormal within normal time reported as normal result.

Normal range: 11-16 seconds
INR patient on stabilized oral anticoagulant therapy N.V 2.0 – 3.5.

Internal Quality control

- Commercial available control are run every day before run the test.

Safety precautions

- Always wear protective cloth when handling samples
- Always wear disposable latex gloves.

Trouble shooting/sources of error

- Washing the humaclot with methlyted spirit
- Re-calibration of the machine

References

- Baker
- Monicah Cheesbrough part 11
- Insert Manual

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by:ALEX T Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE
GPT (GLUTAMATE PYROVATE TRANSAMINASE)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test
L- Alanine + 2 – Oxaglutarate Alanine amino transferase L – glutamate + pyrorate
Pyrorate +NADH + H+ Lactate dehydrogenase L- Lactate + NAO+
The rate in decrease in absorbance due to the axidation of NADH to NAD+ is directly proportional's to the sample.

Clinical significance
IL test ALT/GPT is intended quantitative in ultra diagnostica determination of alanine aminotransferase (ALT or glutamic pyruvate transminase) in human serum.

Equipment
- *Centrifuge, chemistry analyser*

Equipment maintenance
Schedure: Centrifuge lubricant of movable part after every 3 months
General services after 6 months
Chemistry analyser general services after six months
Change the cleaning pad + drying after one month.

Reagent :
- ALT dry (lyophilized powder containing NADH, lactate dehydrogenase
- ALT R1 Containing: buffer, L-alanine and less 0.1% sodium azide
- ALT R2 containing: 2 axoglutarate and less 0.1% sodium azide.

Materials: ***R***eaction curette, sample cups, pastear pipette, pipette filler, blue tips, centrifuge tubes containing clot activator.

Reagent preparation ALT R1 using: reconstitute the powder with the solution invert several times, do not shake and do not allow to foam. Refile the R1 bottle with solution; allow working solution to stand for 30 minutes and mix gently before use.
ALT 2: Ready to use.

Sample Required: Plasma /serum

Sample Collection
- Collect blood using vein puncture into a tube either containing lithium heparin or coated with serum activator.
- Centrifuge to obtain either plasma or serum
- Samples should be analysed as soon as possible after collection or stored at refrigerator (2° - 8°c) for 24 hours.

Procedure

- Feed the computer with patient details.
- Transfer the serum to the sample cup
- Place it into sampling tray
- Return the sample tray in the machine
- Start to analyse the sample.

REPORTING AND RESULT INTERPRETATION

- The result are displayed on the computer screen with 2 different colours thus black and red each with different meaning. Black colour indicates the results are in normal range. Red colour indicate the results are within or below the normal range.

Reference range: Male < 41 u/l
Female < 31 u/l

Internal Quality control

- Normal and abnormal serum control commercially available are run daily before analysing any patient serum sample.
- Patient sample is only run when the normal control serum are within given normal and abnormal control serum are within given range by manufacturer.

Safety precautions

- Always wear protective cloths
- Always wear disposable latex gloves when handling patients samples.
- Do not empty the waste in drain

Trouble shooting/sources of error

- Re – wash the reaction curette
- Re – calibration is done
- Changing reaction curette with absorbent below and above given wavelength given by manufacturer.

References

- Sitati (clinical chemistry book)
- Monicah Cheesbrough vol.1
- Insert Manual from manufacturer kits

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
DIRECT BILIRUBIN

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

Principle of the test
Direct bilirubin + diazotated sulfanilic acid – azobilirubin.
The increase in absorbance due to the formation of azobilirubin is proportional to the direct bilirubin concentration in the sample.
Clinical significance
Direct bilirubin is intended for the quantitative in vitro diagnostic determination of direct (conjugated) bilirubin in human serum using the 1 lab 300 plus chemistry system.
Equipment :

- *Centrifuge, chemistry analyser*

Equipment maintenance
Schedure: Centrifuge lubricant after every 3 months
General services after 6 months
1 lab 300 plus chemistry analyser after six months general service
Wiping the external parts daily with decontaminer eg jik.
Reagent : Hydrocholic acid (R1)

- TD bili R2 – containing, hydrochloric acid, sulfanilic acid
- Sodium nitrite

Materials: centrifuge tubes , pasturer pipettes, micro pipette, pipette filler, test tube rack, reaction curette and sample cups.
Reagent preparation
R1: D-Bili is ready to use. Uncap and place the bottle into the 1 lab 300 plus reagent rank in non-refrigerated area (1-13).
R2: Add 5 drops (0.25ml) sodium nitrite into TD Bili R2 bottle cap and mix by inverting a few times. Then uncap and place into the 1 lab 300 plus reagent rack in the refrigerated area (14 to 33).
Sample Required: Serum and should be protected from exposure to light.
Sample Collection

- Collect venous blood by clean and sterile veinpucture.
- Allow blood to clot properly or using tube containing lithium heparin anticoagulant.
- Centrifuge at 300xg for 3-4 minutes.

Procedure

- Feed the computer with patient details.
- Transfer the serum to the sample cup
- Place it into sampling rack/tray
- Replace the sample rack in the machine
- Start on to analyse the sample.

REPORTING AND RESULT INTERPRETATION

- The result are displayed on the computer screen with different colours thus black and red each with different meaning. Black colour indicates the results are in normal range. Red colour indicates the results are within or below the normal range.

Reference range: 0.0 to 3.4 umol/l

or

0.00 to 0.20mg/l

Internal Quality control

- Normal and abnormal serum control commercially available are run daily before analysing any patient serum sample.
- Patient sample is only run when the normal control serum is within normal and abnormal control serum is within given range by manufacturer.

Safety precautions

- Always wear protective cloths
- Always wear disposable latex gloves when handling patient's samples.
- Do not empty the waste in drain
- Avoid contact with skin and eye (reagents)

Trouble shooting/sources of error

- Re – washing the reaction curette
- Re – calibration is done;
- Changing reaction curette with absorbent below and above given wavelength given by manufacturer.

References

- Sitati (clinical chemistry book)
- Monicah Cheesbrough vol.1
- Insert Manual from manufacturer kits

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
CROSS MATCH (COMPATIBILITY

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure:
Staff Qualified to validate the results:

PRINCIPLE OF THE TEST/METHOD

Donor's red blood cells are reacted with recipients' serum to give rise to a compatible antigen – antibody structure.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- To find out compatible blood to replace blood loss from acute haemorrhage, surgical operations or anaemic patients.
- To detect antibodies in the patients serum which will destroy the donor's cells.

EQUIPMENTS

- Water-bath at 37°c
- Centrifuge
- Test tube
- Pipette
- Pipette filler

EQUIPMENT MAINTENANCE SCHEDULE

Daily, once a week or twice a year depending on the type of the machine.

REAGENTS AND MATERIALS

Antisera, Bovine albumin and AHG are supplied ready to use
0.85% normal saline is prepared by dissolving 0.85g of sodium chloride to 100ml of distilled water.

SAMPLE REQUIRED

Donor's 4% cells suspension
Recipients serum

SAMPLE COLLECTION

Venous blood collection system

PROCEDURE/INSTRUCTIONS

- Take 5 – test tubes and label

1) RT°c (room temperature)
2) Saline 37°c
3) Albumin 37°c
4) AHG 37°c (Anti – Human Globulin)

5) Auto – control (Ac) 37°c

- Into tubes 1-4, add 1 volume of 4% donor cells suspension.
- In to tubes 1-4, add 1 volume of recipients serum.
- In to tube labelled albumin, add 2 drops of bovine albumin.
- Incubate all the tubes at 37°c except tube labelled RT°c which is left at room temperature for ½ -1 hour.

NB: For emergency cases, incubate for 15 minutes

- After incubation wash AHG tube three times with normal saline, discard the saline completely, add AHG and mix.
- Centrifuge all the tubes at 1000rpm for 1 minute

REPORTING AND INTERPRETATION OF RESULTS

Examine all the tubes macro and microscopically for presence or absence of agglutination or even haemolysis.

REFERENCE RANGES/NORMAL VALUES

- Agglutination or haemolysis – incompatible
- No agglutination or no haemolysis - compatible

INTERNAL QUALITY CONTROL PROCEDURE

Cross match can be set by different staff and results are compared.
Also in case of blood transfusion reaction, the test is repeated by a different staff.

METHOD

- Shake the beads bottle thoroughly (for 2 min) before using
- Take a sample of 850ul of the count check beads green into a suitable sample tube. Avoid air bubbles.
- Plug the tube to the sample holder of the flow cytometer
- Use speed 4.0
- Let the system run in count mode until it stops automatically. Count check beads green provide approved bead concentrations for each 10
- Start clean cycle before analysing patient's sample.

TROUBLE SHOOTING/SOURCE OF ERRORS

- Cold agglutination in patient's serum which will clump donor's cells at room temperature.
- Use of penicillin
- Back rial contamination
- Fibrin clot
- Failure to add bovine albumin or AHG
- Failure to add patient's serum
- Over or under centrifugation

INTERPRETATION OF RESULTS

Compatible – if no haemolysis or agglutin
Incompatible – If haemolysed or agglutination noted, hence to repeat test with another pint of blood.

PROCEDURE NOTES

- Even where known, the patient's as well as donors red cell ABO and RH types must be confirmed.
- At all times, patients should receive only blood of the same type.

- Blood for compatibility testing should be selected according to the FEFO policy unless there is a specified request otherwise.

SAFETY PRECAUTIONS

- Wear protective clothing and gloves
- Avoid using broken or cracked test tubes or pipettes

REFERENCES

- Hemovigilance manual
- Introduction to medical laboratory technology by Baker pg 659

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T Signature: .....

Approved by: Signature: ……..

****

STANDARD OPERATING PROCEDURE
UREA

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE

Fixed time analysis. Enyme-couple urease/GLDH methodology.
Urea + H2o urease 2NI4 + 2 HCO3.
NH4 + 2 – axoglutarate +NADH glutamate dehydrogenase < - glutamate + NAD+H2o
The decrease in absorbance at 340nm due to depletion of NADH is proportioned to the urea (urea nitrogen) concentration in the sample.

Clinical significance

- Increase in serum urea (urea nitrogen) can be a result of kidney disfuction or urinary tract obstruction.

Equipment

- Chemistry analyser
- centifuge

Equipment maintenance schedule

Daily calibration
Routine service schedule after 6 months

Reagent preparation

a) Add the entire content of urea – NADH vial into a urea bottle, recap, invert to mix, remove the cup and place into the 1 lab 300 plus reagent rack in the refrigerated area.

Reagent storage stability

- Un opened reagents are stable until the expiration date indicated on the bottle when stored at 2-8°c.

Reagent and materials

Urea: 8 bottles 26 ml containing urease, glutamate dehydrogenase, 2 oxaglutarate, ADP, Tris bustle and less than 0.1% of sodium azide.
Urea-NADH- 8 vials containing; NADH and less than 0.1% of sodium azide.

Specimen

Serum

Specimen collection and preparation

Serum

Urea is stable in stable serum for 3 days at 2°c.-8°c. or 3 months at 20°c.

Reference ranges

Serum – urea 15 to 38mg/dl (2.5-6.4mmo1/l, urea nitrogen – 7 to 18mg/dl

NB: They vary in months, age, gender, diet and geographical areas.

Sample collection

- Selecting the best vein from the arm of the patient.
- Tie the tourniquet
- Sterilize the site using spirit swab
- Withdraw about 3mls of blood and put in Heparin tube.
- Spin at 1000<pm for 2 minutes.
- Specimen is ready for analysis.

Procedure

- Put about 1 ml of the plasma into the sample cup.
- Place in the 1 lab 300 plus rack.
- Follow the machine software commands.

Reporting

- Activity is expressed in either mg/dl or mm/h

Quality Control

- Running the manufactures normal and high pooled plasma.

Trouble shooting/sources of error

Failure to calibrate the machine and run quality controls.

Safety Precautions

- Avoid reagent contact with skin and eyes
- Wear suitable protective clothing
- Don't empty reagent into drains.

Reference

- Monicah Cheesbrough

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by:ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
SODIUM (Na+) ESTIMATION

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019
Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD

Sodium is measured using ion selective electrodes (ISE) where the change in potential difference between reference electrode and the ion selective electrode for the sample is compared with the potential difference for a calibration solution of known composition.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- To detect hypernatraemia or hyponatraemia level in serum

EQUIPMENTS

- Humalyte

EQUIPMENT MAINTENANCE SCHEDULE

Maintenance include daily, non-routine and periodic maintenance.
Daily maintenance involve checking reagent level, cleaning the sample inlet part, conditioning the Na+ electrodes and flow path with serum and also checking the flow time and slope.
Non-routine maintenance is done when instrument generate error codes.
Period maintenance is done twice a year.

REAGENTS AND MATERIALS

Standard A
Standard B
Humalyte Cleaner
Na+ conditioner

REAGENT PREPARATION

Reagents are supplied ready for use

SAMPLE REQUIRED

Serum or Heparin plasma

SAMPLE COLLECTION

By venipunture collection technique

PROCEDURE/INSTRUCTIONS

- Press the analyze key so that the probe remains in the outside position
- Place a sample cup underneath the probe

- Press the analyze key to analyze the sample
- After analysis, the result will automatically be printed and probe return to the inside position.

REPORTING AND INTERPRETATION OF RESULTS

Humalyte will print the result and save the data to memory automatically at the end of each analysis, showing the results on the screen..

REFERENCE RANGES/NORMAL VALUES

- Na+ 135 – 148 mmoI/l

INTERNAL QUALITY CONTROL PROCEDURE

Regular quality control are done on daily basis which include cleaning the fluid path, conditioning and calibration to ensure reliable performance.

TROUBLE SHOOTING/SOURCE OF ERRORS

- Misleading sodium values if blood is collected from arm receiving an intravenous electrolyte or dextrose infusion.

INTERPRETATION OF RESULTS

< 135 mmoI/L indicate hyponatraemia

> 148 indicate hypernatraemia

PROCEDURE NOTES

- Avoiding analysing haemolysed sample
- Avoid stasis during sample collection.

SAFETY PRECAUTIONS

- Wear protective clothing and gloves

REFERENCES

- Medical laboratory manual for tropical countries volume 1 page 488-490.
- Humalyte user manual page 54, 69, 71 and 77.

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
CALCIUM (Ca#)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD

End point analysis, colorimetric methodology based on the reaction of calcium with O-cresolphthalein complexone under alkaline condition to form a purple complex ie:
Calcium + O-Cresulphthalein complexone _____purple colour

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- Calcium measurement are used in the diagnosis and treatment of hypocalcemia and hypercalcemia.

EQUIPMENTS

- Chemistry analyser
- centrifuge

EQUIPMENT MAINTENANCE SCHEDULE

Daily dump dusting, cleaning of curettes, blanking calibration and quality control
Twice a year maintenance

REAGENTS AND MATERIALS

Calcium R1	Test tube rack
Calcium R2	Test tube
Curettes	Pipettes

REAGENT PREPARATION

Reagents are supplied ready for use

SAMPLE REQUIRED

Serum

SAMPLE COLLECTION

By venipunture collection technique

PROCEDURE/INSTRUCTIONS

- Fill in patient's detail in computer screen
- Dispense patients serum into sample cup and place it in sample rack.
- Ensure reagents are positioned in the non-refrigerated area
- Click ok for test to run

REPORTING AND INTERPRETATION OF RESULTS

To increase in absorbance due to purple complex is proportioned to the calcium concentration in the sample.

REFERENCE RANGES/NORMAL VALUES

- Serum: 8.6 – 10.0mg/dl (2.15 – 2.50mmol/L)
- To convert mg/dl to SI units (mmoI/L) multiply by 0.250

INTERNAL QUALITY CONTROL PROCEDURE

Normal and abnormal controls are run daily before patients' analysis.

TROUBLE SHOOTING/SOURCE OF ERRORS

Haemolysed sample

INTERPRETATION OF RESULTS

The higher the concentration of the purple complex, the higher the calcium level.

PROCEDURE NOTES

- Keep the reagents at 15-25ºc for 30 minutes before using.

SAFETY PRECAUTIONS

- In case of reagent contact with eyes, rinse immediately with plenty of water and seek medical advice

REFERENCES

- calcium manual

<u>APPOVAL.</u>

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: ....*

Approved by: *Signature: ………………………..*

****

STANDARD OPERATING PROCEDURE
RETIC COUNT

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD
Reticulocytes are precipitated into a mashwork called reticulum within the cell when stained with basic dyes like brilliant cresyl blue.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST
- Increased or decreased values of reticulocyte count indicate abnormality in the bonemarrow in condition such as haemolytic anaemia, hereditary spherocytosis, and aplastic anaemia/pernicious anaemia.

EQUIPMENTS
- Microscope with ehrlichs eye piece.

EQUIPMENT MAINTENANCE SCHEDULE
Daily dump dusting, occasionally cleaning of oil emmersion and twice a year maintenance.
The microscope is also covered always when not in use.

REAGENTS AND MATERIALS
Brilliant cresyl blue reagent
Small glass tube
Clean slides and spreader
Incubator at 37ºc
Edta tubes
Test tube rack
Drying rack

REAGENT PREPARATION
Weigh the following reagents.
Sodium citrate – 0.6g
Sodium chloride – 0.7g
Distilled water – 100ml
Brilliant cresyl blue gel – 1.0g
Dissolve and filter, label and store ready to use.

SAMPLE REQUIRED
Whole blood in EDTA tube.

SAMPLE COLLECTION
By venipunture collection technique

PROCEDURE/INSTRUCTIONS

- Put 2-4 drops of brilliant cresyl blue in a small test tube
- Add 2-4 drops of patients EDTA whole blood and mix
- Incubate at 37ºc for 15-20 minutes
- Resuspend the cells by gently mixing and make films on glass slides
- Dry and examine the films using ehrlichs eyepiece

NB: No counterstaining or fixing is required.

REPORTING AND INTERPRETATION OF RESULTS

Count at least 1000 red cells and calculate the percentage of reticulocytes present.

Ie $\frac{\text{Total Reticulocyte count seen}}{\text{Total Red cells seen}} \times 100$

RESULT IN PERCENTAGE

Total no. of red cells – 1000

Total no. of retic – 20

Ie $\frac{\text{T. Retic}}{\text{T. Red cells}} \times 100 = \frac{20}{1000} \times 100 = 2\%$

RESULT IN ABSOLUTE RETIC COUNT

Absolute retic count = $\frac{\text{Retic \% x RBC'S COUNT}}{100}$

REFERENCE RANGES/NORMAL VALUES

- Infant (cord blood) – 2-6%
- Adult and children – 0.2 -2.0%

INTERNAL QUALITY CONTROL PROCEDURE

Films with increased, decreased and normal retic count levels are fixed in undiluted leishman's stain for 30 seconds and then counterstained with diluted heishmans stain for 30 seconds, washed off and then dried and kept for reference.

TROUBLE SHOOTING/SOURCE OF ERRORS

- Poorly prepared brilliant cresyl blue stain
- Too thick or too thin film
- Microscope ehrich's eyepiece not in good working condition.

INTERPRETATION OF RESULTS

Reticulocytes appears deep blue meshwork against a relatively unstained background Retic are slightly larger than normal red cells and the more mature the cell, the less reticulum (meshwork) found.

PROCEDURE NOTES

- If ehrich's eyepiece is not available, use a paper diaphragm in the centre cut a small square with sides about 4mm in length and insert into the eyepiece.
- Use new methylene blue which is senior to BCB because it stains more deeply and more uniformly
- The technical staff should be patient in counting.

SAFETY PRECAUTIONS

- Wear protective clothing
- Wear gloves

REFERENCES

- Introduction to medical laboratory technology by baker page 596-597
- Medical laboratory for developing countries by Maurice page 7.17, 7.23 & 7.28

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
SICKLING TEST

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD
HBS and HBSS sickles when exposed to low oxygen tension.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST
- Presence of sickle cells in blood indicates haemoglobinopathy of sickle cell trait (HBAS) or sickle cell anaemia (HBSS).

EQUIPMENTS
- Microscope

EQUIPMENT MAINTENANCE SCHEDULE
Wiping out of oil immersion occasionally and twice a year maintenance
It is also covered always when not in use to avoid dust.

REAGENTS AND MATERIALS
2% sodium meta-bisulphate	Measuring cylinder
EDTA test tube	Distilled water
Clean slides	Paraffin wax or petroleum jelly

REAGENT PREPARATION
2% sodium meta-bisulphate
- Weigh 0.5g of sodium meta-bisulphate powder into a measuring cylinder
- Add 25ml of distilled water.
- This will make 2% solution.

SAMPLE REQUIRED
Whole blood in EDTA Tube
SAMPLE COLLECTION
By venipunture collection technique

PROCEDURE/INSTRUCTIONS
- Put 2 drops of EDTA blood in a test tube
- Add 4 drops of freshy prepared 2% sodium meta-bisulphate (ie 1:2)
- Incubate for 30 minutes at 37ºc under air tight or if slides are used, cover with coverslip and seal with petroleum jelly or paraffin wax.

NB: Hbss disease sickles within 1 hour if negative incubate for as long as 12 Hours for Hbas trait.

- Examine the preparation under microscope mainly at the edges where slight drying often helps the sickling.

REPORTING AND INTERPRETATION OF RESULTS

Sickle cells have long sharp points
Hbss disease – sickles within 1 hour
HbAs trait – sickles after 1 hour (up to 12 hours)

REFERENCE RANGES/NORMAL VALUES

- Normal blood has no sickle cells

INTERNAL QUALITY CONTROL PROCEDURE

Positive slides are prepared using PBF and preserved for reference.

TROUBLE SHOOTING/SOURCE OF ERRORS

Low level of Hbs especially in infants (< 3 months) will give a negative result.

INTERPRETATION OF RESULTS

Presence of sickle cells in blood indicate either sickle cell disease (Hbss) or sickle cell trait (HbAs) type of anaemia.

PROCEDURE NOTES

- Always use fleshy prepared sodium – meta-bisulphate (use the solution for only one day)

SAFETY PRECAUTIONS

- Wear protecting clothing and gloves

REFERENCES

- A medical laboratory for developing countries by Maurice King pg 7.24-7.27

APPOVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
TOTAL CHOLESTEROL

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD

It's an end point analysis ie
Cholesterol esters+O2 cholesterol esterase cholesterol + fatty acids
Cholesterol +O2 cholesterol oxidise cholesr – 4 – en – 3 – one+H2O2
2H2O2 + phenol + 4 – aminoantipyrine peroxidise red quinoneimine + 4H2O

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and of lipid and lipoprotein metabolism disorders.

EQUIPMENTS

- Centrifuge
- Chemistry analyser

EQUIPMENT MAINTENANCE SCHEDULE

Daily dump dusting and twice a year maintenance

REAGENTS AND MATERIALS

Cholesterol reagent curettes
Distilled or deionised water
Pipettes
Test tube
Test tube rack

REAGENT PREPARATION

- Using a volumetric pipette add 23ml of deionised or distilled water to one bottle of 35ml cholesterol.
- Invert 2-3 times to mix the solution then place the bottle into 1 lab 300 plus reagent rack in the refrigerated area (position 14 to 33)

SAMPLE REQUIRED

Serum

SAMPLE COLLECTION

Non-fasting serum specimen through venipunture collection technique
Sample may be stored for 4 days at 2-8°c or 3 months at 17ºc to 23 ºc.

PROCEDURE/INSTRUCTIONS

- Type patient's details in 1 lab computer screen
- Dispense serum in sample cup and position it in a specimen rack
- Ensure reagents are positioned in refrigerated area
- Click ok button for test to run.

REPORTING AND INTERPRETATION OF RESULTS

To increase in absorbance as generated by red dye is proportional to the cholesterol concentration in the sample.

REFERENCE RANGES/NORMAL VALUES

- Serum: 140 -220mg/dl (3.6 – 5.7mmol) normal
- >240mg/dl (>6.2mmol) high
- 200 -23mg/dl (5.2 -6.2mmol) Borderline – high
- <200mg/dl (< 5.2mmol) Desirable

INTERNAL QUALITY CONTROL PROCEDURE

Normal and abnormal controls are run daily before patient's tests are analysed.
Recallibration is done after some days or when a new lot of reagent is used.
Reagent blanking is done daily or before patient assays if the reagent is used less frequently.

TROUBLE SHOOTING/SOURCE OF ERRORS

Highly lipemic or haemolysed sample
Insufficient sample

INTERPRETATION OF RESULTS

The higher the concentration of the red dye, the higher the level of cholesterol level.

PROCEDURE NOTES

- If the sample requires a complete lipid profile, a 12 hours fasting specimen is recommended.
- After daily use of reagent, cap the bottle and place at 2-8 ºc.
- The diluted reagent is stable for 15 days.

SAFETY PRECAUTIONS

- Avoid reagent contact with skin and eyes
- Do not empty reagents in the drain
- Wear suitable protective clothing.

REFERENCES

- cholesterol insert manual

APPOVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
HDL (HIGH DENSITY LIPOPROTEIN)

Document ID Code: *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD

Anti-human B-lipoprotein antibody in R1 binds to lipoprotein other than HDL.
The antigen-antibody complexes formed block enzyme reaction with all lipoprotein except HDL-C when R2 is added.

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- Low HDL – c levels are associated with an increased risk of coronary heart disease.

EQUIPMENTS

- Centrifuge
- Chemistry analyser

EQUIPMENT MAINTENANCE SCHEDULE

Daily dump dusting and twice a year maintenance

REAGENTS AND MATERIALS

HDL R1 curettes
HDL R2
Pipettes
Test tubes
Test tube rack

REAGENT PREPARATION

- HDL – R1 and HDL – R2 are supplied when ready to use.

SAMPLE REQUIRED

Serum

SAMPLE COLLECTION

By venipunture collection technique

PROCEDURE/INSTRUCTIONS

- Fill in patient's details in 1 lab computer screen
- Dispense patients serum in sample cap and position it in sample rack
- Ensure reagents are positioned in refrigerated area
- Click ok button for test to run

REPORTING AND INTERPRETATION OF RESULTS

Hydrogen peroxide produced by enzyme reactions with HDL – c yields a blue coloured complex upon oxidative condensation of the chromogen
The results are automatically displayed on the screen ready to print.

REFERENCE RANGES/NORMAL VALUES

SERUM

- MALE:: 30-75mg/dl (0.78-1.94mmol/l)
- FEMALE: 33-92MG/DL(0.85-2.38MMOL/L
- 35 mg/dl (≥ 0.91mmol/l) desirable
- 60mg/dl (≥ 1.55mmol/l) offer some protection against coronary heart disease

< 35mg/dl (< 0.91 mmol/l) significant independent risk factor for coronary heart disease.

INTERNAL QUALITY CONTROL PROCEDURE

Normal and abnormal controls are run daily before patients tests are analysis.

TROUBLE SHOOTING/SOURCE OF ERRORS

Highly lipemic or haemolysed sample
Insufficient sample

INTERPRETATION OF RESULTS

The concentration of the blue colored complex is proportional to the concentration of HDL –c.

PROCEDURE NOTES

- Samples are stable for up to 4 days at 2-8ºc and up to 3 months at -17 to -23 ºc
- Separate the serum within 3 hours of specimen collection.

SAFETY PRECAUTIONS

- HDL reagents may cause sensitization by skin contact
- Wear suitable protective clothing

REFERENCES

- cholesterol HDL manual

APPOVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
LDL CHOLESTEROL (LOW DENSITY LIPOPROTEINS)

Document ID Code: 411 *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure:
Staff Qualified to validate the results:

PRINCIPLE OF THE TEST/METHOD

When a sample is mixed with LDLR1 (Reagent 1), the protection reagent binds to LDL and protect it from enzyme reaction.
Cholesterol esterase (CHE) and cholesterol oxidise (cd) react with non-LDL lipoproteins
Hydrogen peroxide produced by the enzyme reactions with non LDL cholesterol is decomposed by catalase.
When LDL R2 is added, the protection reagent is removed by sodium azide in this second process, CHE and CO react only with LDL - C

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- High LDL-C levels are associated with an increased risk of coronary heart disease.

EQUIPMENTS

- Chemistry analyser
- centrifuge

EQUIPMENT MAINTENANCE SCHEDULE

Daily dump dusting and twice a year maintenance

REAGENTS AND MATERIALS

LDL R1 curettes
LDL R2
Pipettes
Test tubes
Test tube rack

REAGENT PREPARATION

- LDL – R1 and LDL – R2 reagents are supplied when ready to use.

SAMPLE REQUIRED

Serum

SAMPLE COLLECTION

By venipunture collection technique

PROCEDURE/INSTRUCTIONS

- Type patients details in 1 lab computer screen
- Dispense patient serum in sample cup and position it in specimen rack.
- Ensure reagents are positioned in the refrigerated area (position 14-33).
- Click ok button for test to run.

REPORTING AND INTERPRETATION OF RESULTS

Hydrogen peroxide produced by enzyme reactions with LDL – c yields a blue coloured complex upon oxidative condensation of the chromogen. The blue coloured complex concentration is proportional to the concentration of LDL-C.

REFERENCE RANGES/NORMAL VALUES

SERUM

> 76 – 218mg/dl	(1.97-5.65 mmol/l)	
≤ 130mg/dl	(3.37mmol/l)	Desirable
130-159mg/dl	(3.37-4.12mmol/l)	Border line
≥. 160mg/dl	(>4.14mmol/l)	High risk

INTERNAL QUALITY CONTROL PROCEDURE

Normal and abnormal controls are run daily before patient's tests are analysed.

TROUBLE SHOOTING/SOURCE OF ERRORS

Highly lipemic or haemolysed sample
Insufficient sample

INTERPRETATION OF RESULTS

The higher the concentration of the blue coloured complex, the higher the concentration of LDL-C.

PROCEDURE NOTES

- Store the specimen at 4ºc before analysis
- After daily use of reagents cap bottles and store at 2-8ºc

SAFETY PRECAUTIONS

- LDL –R2 contains sodium azide that may form explosive azides in metal plumbing.
- Use proper disposal procedures.

REFERENCES

- cholesterol HDL manual

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
TRIGLYCERIDES

Document ID Code: 100 *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

PRINCIPLE OF THE TEST/METHOD

It is end point analysis i.e.

Triglycerides Lipoprotein lipase Glycerol + fatty acids

Glycerol +ATP Glycerol Kinase Glycerol – 3 – phosphate + fatty acids

Glycerol – 3 – phosphate +O2 Glycerolphosphate oxidase dihydroxycetate +H2O2
Phosphate

H2O2 + 4 – Chlorophenol + 4 – aminoantipyrine peroxidise RED Quinoneimine +
2H2O

CLINICAL SIGNIFICANCE/PURPOSE OF THE TEST

- Triglycerides measurements are used in the diagnosis and treatment of hyperlipidemia.

EQUIPMENTS

- Chemistry analyser
- centrifuge

EQUIPMENT MAINTENANCE SCHEDULE

Daily dump dusting and twice per year maintenance.

REAGENTS AND MATERIALS

Triglycerides reagent curettes
Sample cup
Pipettes
Test tubes
Test tube rack

REAGENT PREPARATION

- The reagents are supplied when ready to use.

SAMPLE REQUIRED

Serum

SAMPLE COLLECTION

By venipunture collection technique. Separate serum from cells within 2 hours.
Analyse within 24 hours.
Sample is stable for 5-7 days at 2-8ºc or 3 months at 20ºc.

PROCEDURE/INSTRUCTIONS

- Fill in patient's details in 1 lab computer screen
- Dispense patients serum in sample cup and position it in sample rack
- Ensure reagents are positioned in refrigerated area of 1 lab
- Click ok button for test to run

REPORTING AND INTERPRETATION OF RESULTS

The increase in absorbance due to the red dye is proportional to the triglyceride concentration in the sample.

REFERENCE RANGES/NORMAL VALUES

Normal: < 200mg/dl (< 2.26mmol/l)

INTERNAL QUALITY CONTROL PROCEDURE

Normal and abnormal controls are run daily before patient's analysis.

TROUBLE SHOOTING/SOURCE OF ERRORS

Highly haemolysed sample
Insufficient sample

INTERPRETATION OF RESULTS

The higher the concentration of the red dye, the higher the level of triglycerides
Results are automatically displayed on the screen for printing.

PROCEDURE NOTES

- After daily use of reagent, cap the bottle and store at 2-8ºc.
- The reagent is stable for 15 days after opening.

SAFETY PRECAUTIONS

- Do not empty reagent into drains.
- Wear suitable protective clothing

REFERENCES

- Triglyceride insert manual

APPOVAL

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****

STANDARD OPERATING PROCEDURE
MICROSCOPE

Document ID Code: 100 *Version No. 1.0*

Effective Date: 01.02.2018 *Revision Date: 01.10.2018*
Next Revision Date: 02.10.2019

Staff Qualified to perform procedure: LABORATORY TECHNOLOGIST
Staff Qualified to validate the results: LABORATORY TECHNOLOGIST

OPERATING PRINCIPLE AND PURPOSE

A microscope magnifies minute objects making them visible to the eye. The microscope consists of mechanical components, a system of lenses that magnify the specimen placed on the microscope stage, and a light source that illuminates the specimen. The microscope is the most important instrument in the laboratory and is used for examining:

- Blood samples before or after staining
- Bone marrow
- Splenic tissue
- Urine, stool, sputum, body fluids, discharges and skin samples
- Agglutination reactions in blood transfusion medicine.

Installing a microscope

Place the microscope on a firm bench, free from vibration, near an electric power outlet or alternative power source from direct sunlight.

Instruction for using a microscope

1. Always follow carefully the manufacturer's instructions.
2. Connect to the power supply, and switch on the light source.
3. Adjust the eyepieces by sliding them horizontally until both eyes fit comfortably and the two fields merge.
4. Centre the condenser as follows or according to the model of microscope:
    - Swing the x10 objective into position
    - Raise the condenser to the uppermost position
    - Open the iris diaphragm fully
    - Open the light diaphragm to illuminate the whole field
5. Clean and dry the underneath of the glass slide by wiping with cotton gauze.
6. Rotate the nosepiece so the lowest power objective is in position. Slight resistance is felt as the objective moves into the correct position.
7. Place the slide carefully on the stage. Never place the slide on the stage when the x 40 or x100 objectives are in position, to prevent scratching of the lenses.
8. Adjust the illumination:
    - Open the lamp rheostat fully to obtain a bright light
    - Reduce the iris diaphragm to control brightness

9. Focus the specimen by racking the stage carefully upwards with the x10 objective in position. Using the coarse adjustment knob, rack slowly downwards until the image comes into view. Use the fine adjustment knob to focus the image sharply.
10. Swing the x40 and x100(oil immersion) objective into position to examine the specimen in more detail, using the fine adjustment knob to focus.
11. After examination, lower the stage or swing the lowest power objective into position before removing the slide.
12. Wipe oil from the x100(oil immersion) objective and microscope stage using a piece of cotton gauze soaked in absolute methanol, or according to the manufacturer's instructions. Clean with lens tissue.
13. Switch off the microscope, disconnect from power source and cover to protect from dust.

Care and maintenance of a microscope

- Always follow carefully manufacturer's instructions.
- Never remove slides when the x40 and x100 (oil immersion) objectives are in position as this may scratch the lenses.
- Clean the lenses with lens tissue and not cloth or ordinary paper. Do not use methanol on some microscopes as this may dissolve the cement holding the lenses.
- Use a mild soap solution to remove heavy contamination from the instrument surface. Never use acetone.
- At the end of each day, disconnect the power source by switching off the wall socket and remove the plug, or disconnecting the battery terminals.
- Cover the instrument after use.
- To protect against fungus in humid climates, place the microscope in a small cabinet or cupboard that is heated from below by a low watt bulb. The bulb must be left on continuously even when the microscope is not in the cupboard. OR Place the microscope in an airtight plastic bag (made from thick polythene) with self-indicating blue silica gel in a dust –tight linen bag or a dish. Silica gel is blue when active but turns pink when fully saturated with water. To restore its activity, gently heat the silica in an oven or over a flame until the color returns to blue. When the silica gel cools return it to the airtight container. Do not store the microscope in its carrying case or under a plastic hood in humid climates.
- For added security, lock the instrument in a cupboard.
- Protect the microscope from power surges using a voltage stabilizer.
- Replace blown bulbs following the manufacturer's instructions.
- If the equipment is faulty, consult a qualified biomedical engineer.

Troubleshooting a microscope

- Always refer to the operating manual.
- If the microscope fails to switch on, check the electric socket outlet, plug and fuse, or the battery terminals.
- Do not dismantle any part of the microscope. If the microscope is not functioning properly, consults a qualified biomedical engineer.

REFERENCE:

User manual

APPOVAL.

The signature below constitutes the approval of this SOP for use in the laboratory.

Prepared by: ALEX T *Signature: .....*

Approved by: *Signature: ……..*

****