# THE UNITED REPUBLIC OF TANZANIA


## MINISTRY OF HEALTH

# National Medical Standard Operating Procedures for Health Centre Level Laboratory

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**NATIONAL MEDICAL STANDARD OPERATING PROCEDURES FOR HEALTH CENTRE LEVEL LABORATORY**

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# ABBREVIATIONS AND ACRONYMS

For the purposes of this NMLSOP document, these abbreviations and acronyms will apply:

| Abbreviations | Acronyms |
|---|---|
| **AIDS** | Acquired Immunodeficiency Syndrome |
| **AJLM** | African Journal for Laboratory Medicine |
| **AMMP-1** | Adult Morbidity and Mortality Project Phase 1 |
| **AMR** | Antimicrobial Resistance |
| **ANC** | Antenatal Care |
| **APECSA** | Association of Pathologists of East, Central and Southern Africa |
| **APHL** | Association of Public Health Laboratories |
| **APT** | Association of Pathologists of Tanzania |
| **ASCP** | American Society for Clinical Pathology |
| **ASLM** | African Society for Laboratory Medicine |
| **ASM** | American Society for Microbiology |
| **BEmONC** | Basic Emergency Obstetric and Neonatal Care |
| **BMC** | Bugando Medical Centre |
| **BRM** | Biorisk Management |
| **BSC** | Biological Safety Cabinet |
| **BSc** | Bachelor of Science |
| **BSL** | Biosafety Level |
| **BUQ** | Bottom-Up Quantification |
| **CCM** | Chama Cha Mapinduzi (*Ruling Party in the United Republic of Tanzania)* |
| **CD4** | Cluster of Differentiation 4 |
| **CDC** | Centers for Disease Control and Prevention |
| **CDs** | Cluster of Differentiations |
| **CEmONC** | Comprehensive Emergency Obstetric and Neonatal Care |
| **CEPD** | Continuing Education and Professional Development |
| **CHF** | Community Health Financing |
| **CHMT** | Council Health Management Team |
| **CHSB** | Council Health Service Board |
| **CHWs** | Community Health Workers |
| **CLS** | Community Laboratory Services |
| **CLSI** | Clinical and Laboratory Standards Institute |
| **COPECSA** | College of Pathologists, East, Central and Southern Africa |
| **CPD** | Continuing Professional Development |
| **CPL** | Central Pathology Laboratory |
| **CTRL** | Central Tuberculosis Reference Laboratories |
| **DDHCTSU** | Director, Diagnostic and Health Care Technical Services Unit |
| **DED** | District Executive Director |
| **DHs** | District Hospitals |

| Abbreviations | Acronyms |
|---|---|
| DLTs | District Laboratory Technologists |
| DMO | District Medical Officer |
| DO | Data Officer |
| DP | Development Partner |
| DQA | Data Quality Assurance |
| DTS | Dried Tube Specimen |
| EAC | East African Community |
| EAPHLN | East African Public Health Laboratory Network |
| ECSA | East, Central, and Southern African |
| eLIS | electronic Laboratory Information System |
| eLMIS | Electronic Logistics Management Information System |
| EOC | Emergency Operations Centre |
| EQA | External Quality Assessment |
| FBO | Faith Based Organisations |
| FYDP | Five Years' Development Plan |
| GCLA | Government Chemistry Laboratory Agency |
| GHSA | Global Health Security Agenda |
| GOT | Government of Tanzania |
| HCRF | Health Commodity Revolving Fund |
| HCTS | Health Care Technical Services |
| HIV | Human Immunodeficiency Virus |
| HLI | Health Links Initiative |
| HLPC | Health Laboratory Practitioners' Council |
| HMIS | Health Management Information Systems |
| HMTs | Health Management Teams |
| HSSP IV | Health Sector Strategic Plan IV |
| HSSP V | Health Sector Strategic Plan V |
| I-TECH | International Training and Education Centre for Health |
| IATA | International Air Transport Association |
| ICAP | International Center for AIDS Care and Treatment Programs |
| IDSR | Integrated Disease Surveillance and Response |
| IHR | International Health Regulations |
| IMPACT | Information Mobilise for Performance Analysis and Continuous Transformation |
| IMTU | International Medical and Technological University |
| ISBN | International Standard Book Number |
| ISO | International Organization for Standardization |
| IT | Information Technology |
| KCMC | Kilimanjaro Christian Medical Centre |
| KIU | Kampala International University |
| KPI | Key Performance Indicator |
| LEMM | Laboratory Equipment Management Module |
| LIO | Laboratory Information Officer |
| LIS | Laboratory Information System |
| LMIS | Logistic Management Information System |

| Abbreviations | Acronyms |
|---|---|
| LO | Logistic Officer |
| LQA | Laboratory Quality Assurance |
| MDG | Millennium Development Goals |
| MeLSAT | Medical Laboratory Scientists Association of Tanzania |
| MMAM | Mpango wa Maendeleo ya Afya ya Msingi |
| MNCH | Mother and Neonatal Child Health |
| MoH | Ministry of Health |
| MOU | Memorandum of Understanding |
| MS | Marketing Surveillance |
| MSc | Master of Science |
| MSD | Medical Stores Department |
| MSD | Medical Stores Department |
| MTBDR | Mycobacterium Tuberculosis Drug Resistance |
| MTEF | Medium Term Expenditure Framework |
| MUHAS | Muhimbili University of Health and Allied Sciences |
| NACP | National AIDS Control Programme |
| NACTE | National Council for Technical Education |
| NBTS | National Blood Transfusion Services |
| NCDs | N0n-Communicable Diseases |
| NGO | Non-Governmental Organisation |
| NHLS | National Health Laboratory Services |
| NHLSP | National Health Laboratory Strategic Plan |
| NIMR | National Institute for Medical Research |
| NMCP | National Malaria Control Programme |
| NMLSSP III | National Medical Laboratory Services Strategic Plan III |
| NPHL | National Public Health Laboratory |
| NPHLA | National Public Health Laboratory Agency |
| NRL | National Reference Laboratory |
| NSCLQS | National Sub-Committee on Laboratory Quality System |
| NSGHLS | National Standard Guidelines for Health Laboratory Services |
| NSGRP | National Strategy for Growth and Reduction of Poverty |
| NSLQMS | National Sub-committee for Laboratory Quality Management Systems |
| NTDs | Neglected Tropical Diseases |
| NTLP | National Tuberculosis and Leprosy Programme |
| PEPFAR | President's Emergency Plan for AIDS Relief |
| PHDR | Poverty and Human Development Report |
| PHIA | Population-based HIV Impact Assessment |
| PHLB | Private Health Laboratory Board |
| PHLS | Public Health Laboratory Services |
| PLHIV | People Living with HIV and AIDS |
| PO-RALG | President's Office-Regional Administration and Local Government |
| POC | Point of Care |
| PPM | Planned Preventive Maintenance |

| Abbreviations | Acronyms |
|---|---|
| PPP | Public Private Partnership |
| PT | Proficiency Testing |
| QA | Quality Assurance |
| QC | Quality Control |
| QMS | Quality Management System |
| QO | Quality Officer |
| RAS | Regional Administrative Secretary |
| RBF | Results-based Financing |
| RFM | Result Framework Matrix |
| RHMT | Regional Health Management Team |
| RLQA | Regional Laboratory Quality Assurance |
| RLTs | Regional Laboratory Technologists |
| RMO | Regional Medical Officer |
| RRH | Region Referral Hospital |
| RTQII | Rapid Testing Quality Improvement Initiative |
| SADC | Southern African Development Community |
| SADCAS | Southern African Development Community Accreditation System |
| SCM | Supply Chain Management |
| SD | Standard Deviation |
| SDG | Sustainable Development Goals |
| SLIPTA | Stepwise Laboratory Improvement Process Towards Accreditation |
| SLMTA | Strengthening Laboratory Management Toward Accreditation |
| SO | Safety Officer |
| SWOC | Strength Weakness Opportunities and Challenges |
| TA | Technical Assistance |
| TB | Tuberculosis |
| TCU | Tanzania Commission for Universities |
| THPS | Tanzania Health Promotion Support |
| TIKA | Tiba kwa Kadi |
| TMDA | Tanzania Medicine and Medical Devices Authority |
| TOR | Terms of Reference |
| TOT | Trainer Of Trainee |
| TSPAS | Tanzania Service Provision Assessment Survey |
| URT | United Republic of Tanzania |
| WHO | World Health Organization |
| ZACDS | Zonal Advisory Committee on Diagnostic Services |

# TERMS AND DEFINITIONS

For the purposes of this NMLSOP document, these terms and definitions will apply:

| Terms | Applicable Definitions |
|---|---|
| **Demand** | Refers to consumers' desire to acquire the services, and the willingness to pay for it. |
| **Access** | Refers to the ability of people to reach places and services and the ability of places to be reached by people and goods |
| **Quality** | Refers to the degree to which a set of inherent characteristics of products or service fulfils requirements |
| **Resilience** | Refers to the process of adapting well in the face of adversity, trauma, tragedy, threats, or significant sources of stress - such as relationship problems, serious health problems, or workplace and financial stressors |
| **Accountability** | Refers to responsibility of an individual to complete assigned tasks and to perform the duties required by their job |
| **Learning** | Refers to the process of acquiring new understanding, knowledge, behaviours, skills, values, attitudes and preferences |
| **Operational plan** | Refers to a comprehensive and actionable plan that defines how team's functions and activities contribute to an organisations overall business goal |

# FOREWORD

This is the first National Medical Laboratory Standard Operating Procedure (NMLSOP).

The document outlines the Standard Operating Procedures that the laboratory tests are to be performed in various facilities during the period of one year's towards strengthening the provision of quality medical laboratory services. The plan was developed through consultative process involving various stakeholders within the Ministry of Health and other Ministry of the government of Tanzania.

This plan describes the background of medical laboratory sciences, as well as the context in which it was established in the country. It also features the current roles, functions and structure of the organisation. Furthermore, contents of the plan include; stakeholders' analysis, strengths, weaknesses, opportunities, and challenges that face medical laboratory services. In identifying these elements, SWOC and Political, Economic, Social, Technological, Environmental and Legal (PESTEL) analysis were used.

The NMLSOP have been developed in line with the other medical laboratory's technical procedures. This NMLSOP is presented in seven (7) chapters set in a path to overcome the weaknesses and challenges as well as proving a tool to take advantage of organisation's strength in exploring the existing opportunities. The SOPs include purpose, scope, responsibilities, principle and many steps. This NMLSOP is expected to be reviewed annually from January 2024 to January 2025.

Successful implementation of this NMLSOP depends on the availability of technical and financial resources to coordinate its execution. Hence, all stakeholders should be aware of their roles and responsibilities at the national, regional, and council levels outlined in this strategy. It is expected that, MoH through the Director, Diagnostic and Health Care Technical Services Unit (DDS) will provide the required leadership and guidance.

**Dr. John A. K. Jingu**
**PERMANENT SECRETARY**

# ACKNOWLEDGEMENTS

The National Medical Laboratory Standard Operating Procedure (NMLSOP) is a product of dedicated efforts and contributions of various stakeholders. The MoH acknowledges the contribution of PO-RALG, Development and Implementing Partners, Non-Government Organisations (NGOs), Institutions or facilities (National Hospitals, Zonal Hospitals, Specialised Hospitals, Regional Referral Hospitals, District Hospitals, Health Centers, Dispensaries), Regulatory Agencies (Health Laboratory Practitioner's Council and Private Health Laboratories Board), Programmes (NASHCoP, NTLP, NMCP, NBTS) and Individuals towards improving the quality of health care service delivery through improved medical laboratory services.

In particular, the MoH would like to thank the Global Fund (GF) and Centres for Disease Control and Prevention (CDC) for their financial and technical support through the consultancy in development of this strategic plan. Special appreciations are extended to technical experts and individuals for their active participation and constructive inputs and comments provided in reviewing this guideline (**ANNEX 01**).

Our appreciations go to the Director of diagnostic and Health Care Technical Services Unit (DDS) Dr Alex S. Magesa for steering up the whole process; Acting Head of Laboratory Services (Ag. HLS) Mr. Reuben S. Mkala for field coordination; and to all laboratory technical team or individuals who played a pivotal role in developing this NMLSOP by participating in the consultation workshops, meetings, providing relevant information and offering their expert opinion when consulted. Last but not the least; appreciations go to David Ocheng, who facilitated the whole

**Prof.Tumaini J. Nagu**
**CHIEF MEDICAL OFFICER**

# EXECUTIVE SUMMARY

In developing this National Medical Laboratory Standard Operating Procedure (NMLSOP) for the period 2024-2025 consideration has been made on a number of National and International guiding and operational guidelines. National Standard for Medical Laboratories (2017); Health Care Technology Policy Guideline (2004); Operational Plan for the National Laboratory System to Support HIV and AIDS Care and Treatment (2005); National Laboratory Quality Assurance Framework to Support Health Care Interventions (2007); Standard Guidelines for the Facilities and Operations of Forensic Bureau Laboratory (2008); International Health Regulations (IHR) of 2005; Global Health Security Agenda (GHSA) 2024 Framework; and the Ruling Party Election Manifesto 2020.

The main objective of this SOPs is to enable the laboratories at all levels to effectively and efficiently carry out their core functions of laboratory tests as stipulated by their mandate, strategically coordinate and allocate the available resources by prioritising functions with effective impact in line with the National Health Policy (2007) and the Sustainable Development Goals (SDGs).

There are four chapters in this document. Chapter one provides; a brief introduction and background information, justification and objectives for the NMLSOP.

This National Medical Laboratory Standard Operating Procedure has four strategic directions:

1. Enhanced conducive Political, Social and Economic environment for Medical Laboratory services (Resilience);
2. Improved and strengthened Medical Laboratory Diagnostic services (Access);
3. Heightened effective collaboration and partnerships for coordinated action (Demand);
4. Strengthened Quality Management systems, Surveillance and Monitoring and Evaluation (Quality).

For each objective above, strategies, targets, activities and performance indicators were derived. The NMLSOP Matrix specifically describes the sequence of objectives, strategies, targets, activities and performance indicators are considered the guiding framework for the development and implementation of annual operational plans.

## Scope

The scope of this National Medical Laboratory Standard Operating Procedure (NMLSOP) is to provide a step-by-step instruction for performing laboratory tests.

## Primary Beneficiaries

The primary beneficiaries are Laboratory Practitioners' who perform examination of biological samples in the laboratories and non-laboratory testers performed specialised laboratory tests at point-of-care testing sites.

## Secondary Beneficiaries

The Secondary users of this document will include but not be limited to:

Policy makers, medical care managers and administrators, medical devices regulatory authorities, medical laboratories, intervention programmes, Regional Health Management Teams and Council Health Management Teams, zonal medical equipment workshops, biomedical engineers, healthcare technical services and end-users engaged in strengthening the quality of medical diagnostic services in the country.

Additionally, other beneficiaries include: Development and Implementing Partners who provide technical assistance to support the Government of Tanzania (GOT) to implement the medical laboratory agenda, public and private medical institutions and laboratories, and higher learning medical laboratory training institutions, prospective accrediting agencies, medical professional bodies and clinical laboratory medicine.

The objective of NMLSOP is to ensure that Laboratory personnel implement National Medical Laboratory Standard Operating Procedure for continuity of quality medical diagnostic laboratory services as needed by the clients.

# CHAPTER 1: SAMPLE COLLECTION

## 1.0 GENERAL CONSIDERATIONS

The collection of samples for laboratory tests from patients consists of following steps:

i. Documentation/Registration of the patient
ii. Collection of sample
iii. Dispatch of sample to respective department

### *1.1.1 Handling of Biological Samples*

Laboratory staffs are often confronted with the problem of handling of biological samples from patients. The following must be observed for personal protection;

i. All biological samples must be considered hazardous and infected.
ii. Wearing of personal protective equipment (PPEs),
iii. Exercising due care to prevent spillage/splashes while transferring blood to containers from syringe,
iv. The sample containers be labelled with adequate information.

### *1.1.2 Samples for Culture*

i. As far as possible samples for culture should be obtained before administration of antimicrobial agents.
ii. If it is not possible, then the laboratory should be informed about the therapeutic agent(s) so that this fact is considered before issuing laboratory report.
iii. Material should be collected from the appropriate site where the likelihood and possibility of isolation of suspected organisms is high.
iv. Sometimes patient's active participation is necessary for sample collection
v. (sputum or urine), so he/she should be instructed properly and accordingly.
vi. Sufficient quantity of samples is to be collected to permit complete examination.
vii. Samples are to be placed in sterile containers.
viii. Some samples are directly collected in culture media. Contact laboratory if such collection is required.
ix. Proper labelling of samples should always be done with patient's name, test type, date and site of collection etc.
x. The relevant clinical information is to be recorded on the request form.
xi. Any condition, circumstances or situation that will require special procedures should also be noted on the request from.
xii. Samples should be collected during working hours except in emergency, so that the services of qualified microbiologist will be available to directly supervise processing of the sample.

xiii. The most appropriate samples for isolation of viral, chlamydial or rickettsial agents depend on the nature of the illness.
xiv. The material should be collected as early as possible in the acute phase of the disease, because these agents tend to disappear relatively rapidly after the onset of the symptoms.
xv. Vesicle fluid is preferably collected in a syringe or capillary pipette and immediately diluted in an equal volume of skimmed milk or tissue culture medium.
xvi. All samples for viral culture should be frozen and stored at -70°C until culture is initiated.

### *1.1.3 Dispatch of Samples from Reception to the Laboratory Sections*

i. Match the containers and respective request forms, number them and enter in the dispatch register/computer.
ii. Verify while handing over/taking away to respective department of the laboratory.
iii. Notify the concerned department about urgent and special tests.

### *1.1.4 Sample Transportation*

i. Exterior of the container should not be soiled/contaminated with the samples.
ii. Sufficient absorbent materials must be used to pack the sample, so that it absorbs the spilled liquid in case of leakage/breakage during transit to reference/referral laboratory.
iii. Sample containers must be leak proof and unbreakable. Plastic containers are preferred.
iv. Samples must be promptly delivered to the laboratory for valid results.
v. If applicable, appropriate transport media should be used.
vi. Samples are to be refrigerated or incubated at 37°C, as the case may be, if there is a delay in transport of samples to laboratory.
vii. An appropriately filled request form should always accompany all samples to guide the pathologist/ health laboratory practitioner in selection of suitable media or appropriate technique.

## 1.2 COLLECTION OF BLOOD SAMPLES

Consider the following recommended order of veins during blood drawing;

- Median cubital vein (first choice),
- Cephalic vein (second choice),
- Basilic vein (third choice).

### *1.2.1 Blood sample for serology*

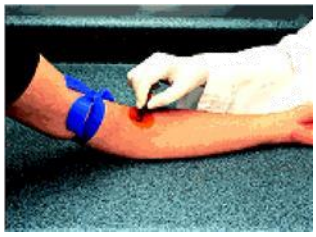
i. Serological tests are required in most of the bacterial, viral and parasitic diseases.
ii. A clotted blood sample is preferred.
iii. A vacuum collection system is both convenient as well as reliable.

iv. Wherever applicable, paired samples are to be collected during acute and convalescent phases of illness in certain viral and other infections to document a diagnostic rise in antibody titre.
v. Protect blood samples from extremes of heat and cold during transport.
vi. Whole blood is to be stored at 2-8°C.
vii. Serum can be frozen at -20°C or lower temperature for long term storage.

### *1.2.2 Blood sample for culture*

i. Make sure you have the appropriate media for blood culture, as the media may vary depending upon the type of pathogen suspected.
ii. Wash the hands with soap and water and wear sterile gloves.
iii. Withdraw the blood following the procedure described on the procedure for collection of venous blood. Change needle before injecting the blood into the culture bottle.
iv. Thoroughly clean the rubber bung of the culture bottle with iodine solution and inject an amount of blood equal to 10% of the volume of medium (for 30 ml medium 3 ml blood and for 50 ml medium, 5 ml blood is needed).
v. After the needle has been removed, the site should be cleaned with 70% alcohol/spirit swab again.
vi. Don't store the containers and caps separately.
vii. Blood obtained for culture of suspected anaerobes should not be exposed to air in any way.

### *1.2.3 Venous blood*



i. Welcome and greet the patient**,** introduce yourself**.**
ii. Make the patient sit comfortably on the phlebotomy chair. iii. Identify the patient by asking his/her particulars and compare them with the request form.
iii. Check the request form for the requested investigations and Inform the patient about the samples to be collected.
iv. Where possible, ask the patient to remove any tight - fitting sleeved clothing, or to roll up loose sleeves.
v. Lable the containers before obtaining sample.
vi. Select syringe of appropriate size so that the quantity of blood required can be obtained in single prick. If multiple or high volume of samples is required, use a butterfly needle or a canula.
vii. Select appropriate vein (preferably antecubital) from forearm. Cleanse the skin over the venepuncture site in a circle approximately 5 cm in diameter with 70% alcohol/spirit swab and allow to air dry, do not blow.
viii. If the sample is to be collected for blood culture then skin is to be thoroughly sterilised, following the procedure as follows:
    a. Starting in the centre of a circle

b. apply 2% iodine (or povidone-iodine) in everwidening circles until the entire chosen area has been saturated with iodine.
c. Allow the iodine to dry on the skin for at least 1 min.
d. Completely remove the iodine with 70% alcohol/spirit swab following the pattern of application.

viii. Apply a tourniquet tight enough to obstruct venous flow only and relocate the vein to be punctured but don't touch the proposed site of needle entry or the needle itself. Ask the patient to clench the fist to make the veins prominent. If the vein is not visible, palpate it with fingers. In case the veins of forearm are not visible/palpable, other sites such as dorsum of the hand may be selected.

ix. With bevel up, insert the correct needle size on the veins at an angle between 15 - 30o then draw the blood into the appropriate collection tubes, (make sure the patient arm in a downward position to prevent reflux).

x. Mix immediately after drawing each tube that contain an additive by gently inverting the tube 8 - 10 times. Do not mix vigorously in order to avoid haemolysis.

xi. Release the torniquet from the patient, Withdraw the needle and apply pressure to the puncture site using dry cotton balls/gauze pad. Do not withdraw the piston too forcefully as it can collapse the vein and it may cause frothing/ haemolysis of the sample.

xii. Apply pressure with thumb on antiseptic swab at puncture site for 2 - 4 min till the blood ooze stops.

xiii. If syringe was used, safely remove the needle from the syringe before distribution.

xiv. The blood from syringe is distributed to appropriate, labelled containers.

**NOTE:**

In case of multiple blood sample collection, consider the following recommended order of draw;

i. First tube blood culture,

ii. Second tube: non-additive tube (e.g. red stopper),

iii. Third tube: coagulation tube (e.g. blue stopper),

iv. Last tube: additive tube (e.g. lavender or green tube).

### *1.2.4 Capillary Blood Collection*

i. Wear sterile gloves
ii. Assemble All Collection Tools
iii. Clean the ring fingertip with 70% isopropyl alcohol swab or 70% spirit starting the middle and leave outward to prevent contaminating the area,
iv. Leave the site to air-dry,
v. Hold the finger firmly place the new sterile lancet device at the site on the finger,

vi. Wipe first drop of blood with a clean dry gauze or cotton wool,
vii. Collect the sample with the second drop of blood using appropriate sample collection devices such as blood capillary tube,
viii. Apply pressure with a clean dry gauze pad or cotton wool until bleeding stop,
ix. Transfer the collected blood sample into appropriate sample container or testing devices.

### *1.2.5 Procedure for Performing Neonate Capillary Blood Collection*

i. Use the most medial or lateral portions of the planter surface of the heel Limit the depth of the puncture wound by using an automated lancet.
ii. Only consider using the whole plantar surface of the foot (using automated lancets of 2.2mm in length or less) for neonates over 33 weeks' gestation if they are having multiple/frequent heel pricks
iii. Position the neonate: ensure the foot is lower than the body.
iv. Choose a puncture site – do not use a previous puncture site.
    a. Clean the heel site (i.e. gauze and water) if the foot appears unclean (e.g. faecal material).
    b. Encircle the foot with the palm of the hand and the index finger.
    c. Make a quick puncture with the automated lancet device
    d. Wipe off the first drop of blood with a gauze swab
    e. Collect the sample with the second drop of blood using any of the collection devices such as slides or rapid test
    f. Apply pressure with a clean dry gauze pad until bleeding stop

## 1.3 COLLECTION OF URINE SAMPLES

Urine samples are collected for routine and culture examinations to diagnose urinary tract infections (UTIs), both lower UTIs (cystitis-infection of the bladder) and upper UTIs (pyelonephritis-infection of the kidney). Unlike most other cultures, colony counts are done on urine samples to determine the number of organisms present in the sample. Generally, >100,000 organisms/ml of a single isolate indicate an individual has a UTI. However, mixed UTIs do occur and some individuals with UTIs will have counts lower than 100,000 organisms/ml.

Most organisms that cause UTIs are normal enteric flora, including *E. coli, Proteus, Klebsiella*, *Enterobacter*, and *Enterococcus* species. In young, otherwise healthy, sexually active females, *Staphylococcus saprophyticus* can be found to be the cause of UTI.

UTIs caused by *Proteus* species can be complicated by the formation of urinary calculi or stones. The large amount of urease that *Proteus* produces can alkalinize the urine.

If there are minerals such as phosphates or carbonates in the urine, the alkaline pH can cause them to precipitate out and form stones.

### 1.3.1 TYPES OF URINE SAMPLE

**A. First morning urine sample**

It provides concentrated urine as the bladder incubated it the whole night. It is best for nitrite, protein, good for microscopic examination and culture and sensitivity. The casts may have deteriorated and bacteria may affect true glucose reading.

**B. Random (routine) urine sample**

It is the most common type and most convenient sample. It is good for observing physical characteristics, chemical analysis and identification of casts, crystals and cells.

**C. Second-voided urine sample**

The first morning sample is discarded and second sample is collected. Formed elements remain intact.

**D. Mid stream (clean catch) urine sample**

The portion of urine that does not contain the first and last portions of the sample.

**E. Post-prandial**

It is collected after meal (usually after 2 hours). It is good for glucose and protein estimation. Urine sugar testing now has limited diagnostic or prognostic value.

**F. Timed sample**

It is a combination of all voiding over a length of time. Two-hour sample is good for urobilinogen and 24-hour sample is good for quantitative urinary components estimation. Timed urine samples are collected in dynamic function tests.

**G. Foley catheter**

Disinfect a portion of the catheter with alcohol, puncturing the tubing directly with a sterile syringe and needle and aspirate the urine. Place urine in a sterile container, it should never be collected from drainage bag.

**H. Suprapubic urine**

Urine sample collected by suprapubic aspiration and cystoscopy.

#### *1.3.1.1 Procedures for Collection of Urine Sample*

Urine sample is often collected by patient him/herself. Therefore, the patient needs to be properly instructed to have correct sample collection. An uncontaminated midstream urine (MSU) sample is the best and following methods are to be used for its collection:

**A. Females**

i. Wash the genital area thoroughly with - Clean water (may be omitted for urine Routine Examination).
ii. With two fingers of one hand, hold the outer folds of vagina (labia) apart. With the other hand, rinse the area from the front to the back with running tap water.
iii. Start urination so that the stream of urine should flow without touching the skin. After a few moments, place a sterile container under the stream of urine. Remove it from the urine stream the moment required amount of urine is collected.
iv. Secure and tighten the cap on the container.

**B. Males**

i. Wash the genital, area thoroughly with Clean water (may be omitted for urine Routine Examination).
ii. Start urination and after a few moments, place a sterile container under the stream of urine. Collect the required amount of urine and remove the container from urine stream.
iii. Secure and tighten the cap.

**C. Infants, uncooperative and debilitated patients**

i. Plastic bags may be attached after careful and thorough washing of genital area.
ii. The bags should be watched so that they can be removed immediately after patient has passed the urine.
iii. If the patient has not voided urine within 30 min the collecting bag is removed.
iv. Patient needs to be re-scrubbed and a new collection device is to be attached.

**D. Urine collection for *Mycobacterium tuberculosis***

i. Three consecutive early morning samples (>90 ml each) collected in sterile container are superior to 24h collection.
ii. Boric acid (1.6%) is used as preservative in case of 24h urine collection in exceptional situations e.g., when patient cannot report daily for sampling.
iii. Suprapubic aspiration in ward by a doctor is preferred in catheterised patients.

## 1.4 FAECAL SAMPLES COLLECTION PROCEDURE

Faecal samples are collected for routine and culture examinations to find the causative agent of infectious diarrhoea. Rectal swabs are often helpful in identifying the cause of acute bacterial diarrhoea when stool sample cannot be collected readily.
It is important to remember that there are many causes of diarrhoea other than infectious agents such as metabolic disorders, certain drugs, and food intolerances (allergies). In a routine stool culture, the following organisms are often isolated: *Salmonella* species, *Shigella* species and *Vibrio cholera.* Viruses and parasites are also common causes of diarrheal disease.

### *1.4.1 Procedure for Collection of faecal samples*

i. Faeces should be passed directly into a clean, waxed cardboard container that is fitted with a tight cover.
ii. Avoid contact with residual soap/detergent, disinfectant or urine in the bedpan.
iii. Faeces obtained are transferred to another clean, wide mouthed and screw capped container. The sample should include any pus, blood, mucus or formed elements that may have passed with stool.
iv. Sample (~1 ml) is added to 10 ml sterile alkaline peptone water in suspected cholera cases.
v. If viral infection is suspected, faeces are extracted with sterile buffered saline. Faeces (~1 ml) are mixed with 9 ml sterile buffered saline, allowed to sediment for 30 min (or centrifuged). The supernatant is transferred to a sterile container, frozen and kept below -40°C until processed.

## 1.5 COLLECTION OF BODY FLUIDS

The primary body fluid that are collected for routine and culture examinations includes; cerebrospinal fluid (CSF), joint fluid, pleural fluid, and peritoneal (ascites) fluid.

### *1.5.1 Cerebrospinal fluid (CSF)*

i. CSF routine and culture examinations are performed to diagnose meningitis due to Viruses, fungi, and bacteria. Acute bacterial meningitis (ABM) is a medical emergency.
ii. The age and immune status of the patient influence the type of bacterial pathogen most likely to cause ABM:
iii. Neonates 0 – 2 months; *E. coli, Streptococcus agalactiae* - Group B streptococci, *Listeria monocytogenes*
iv. months - 2 years; *Haemophilus influenza* and *Neisseria meningitidis*
v. Older than 2 years; *Neisseria meningitides* – most common in children and young adults, *Streptococcus pneumoniae* – most common in older adults

#### 1.5.1.1 Procedures for Collection of CSF samples

CSF is normally collected from sub-arachnoid space of spinal cord at lumber level by puncture with a long needle. A physician in the ward under strict aseptic conditions performs the procedure.

i. Sample shall be collected in 2-4 ml quantities in 3-4 sterile screw capped bottles that are serially numbered and must be sent to the laboratory immediately.
ii. In case CSF is to be cultured for *M. tuberculosis* then at least 5 ml sample is needed. CSF shall be tested as soon as it arrives in the laboratory.
iii. CSF in the first bottle is sometimes contaminated with blood and should be kept aside.
iv. Fluid from second bottle is used for routine tests while fluid from third bottle is used for bacterial culture etc.
v. If tuberculous meningitis is suspected, 4th bottle is kept in refrigerator undisturbed to see whether a pellicle or coagulum forms.

***NB: CSF must never be refrigerated (if for bacterial culture as it kills H. Influenzae) and should be kept at 37°C.***

#### *1.5.1.2 Body Effusions (Exudates and Transudates)*

An effusion is fluid which collects in a body cavity or joint. Fluid which collects due to an inflammatory process is referred to as an **exudate** (needs investigations) and that which forms due to a non-inflammatory condition is referred to as a **transudate** (needs no microbiological investigations). Effusions include; pleural, pericardial, synovial, peritoneal, and hydrocele fluids.

#### *1.5.1.3 Pleural and Pericardial Fluids*

Main purpose of testing is to ascertain their transudative or exudative nature and to find a causative organism if an infective process is indicated. See sputum sample for list of organisms that can be isolated from pleural samples.

#### *1.5.1.4 Peritoneal Fluid - Ascites*

The common indications for paracentesis are ascites of unknown origin, suspected intestinal perforation, haemorrhage or infarct, infections like tuberculosis, complications of cirrhosis (spontaneous bacterial peritonitis) and suspected intraabdominal malignant disorders.

#### *1.5.1.5 Joint fluid (synovial fluid)*

Joint fluid cultures are performed to diagnose septic arthritis (most cases of arthritis are NOT infectious; they are due to strain on a joint or immunological diseases).

The three most common causes of septic arthritis are: *Staphylococcus aureus, Neisseria gonorrhoea,* and Coagulase-negative staphylococci in patients with joint replacement prosthetics

#### *1.5.1.6 Hydrocele Fluid*

Usually from the sac surrounding the testes. Occasionally *Wuchereria bancrofti* microfilariae and rarely *Brugia* species can be found in hydrocele fluid.

##### *1.5.1.6.1 Collection of aspiration fluids (effusions)*

i. Collection of synovial, pleural, pericardial, peritoneal, or hydrocele fluid is carried out by a medical officer or competent nurse.
ii. Label each container with the date and the patient's identifiers
iii. After aspiration, aseptically dispense the fluid into 3 tubes as follows:
    - 5 to 10 ml is in a sterile tube for microbiological examination.
    - 5 ml in anticoagulant (heparin, trisodium citrate or EDTA) for estimation of cell count and protein concentration.
    - 2-3 ml in a plain tube and allowed to clot (normal fluid does not clot).
iv. If the sample cannot be examined immediately, fluid should be frozen and stored at -70°C until examined**.**

COLLECTION OF GENITAL SAMPLES

i. Indicated for the diagnosis of bacterial sexually transmitted diseases, primarily gonorrhea (GC) or non-gonococcal cervicitis or urethritis (NGU). The most common cause of NGU is *Chlamydia trachomatis.*
ii. immunological and molecular tests for the diagnosis of chlamydial infections includes (PCR, DNA probes, etc.) .
iii. Vaginal secretions are also sent to the laboratory for the diagnosis of vaginitis.
iv. The diagnosis of vaginitis can be made with a wet mount – yeast, trichomonas, bacterial vaginosis (BV), culture – yeast, or Gram stain – yeast and BV.
v. Urethritis, cervicitis: *Neisseria gonorrhoeae, Chlamydia trachomatis,* Other agents of NGU
vi. Vaginitis: *Candida albicans, Trichomonas vaginalis and* BV

### *1.5.2 Urethral swabs*

Possible pathogens; *Neisseria gonorrhoea, Chlamydia trachomatis,* and *Trichomonas vaginalis*.

### *1.5.3 Collection* of urethral discharge from male patients

i. Cleanse around the urethral opening using a swab moistened with sterile physiological saline.
ii. Gently massage the urethra from above downwards.
iii. Using a swab, collect a sample of discharge.

iv. Make a smear of the discharge on a microscope slide by gently *rolling* the swab on the slide. This will avoid damaging pus cells which contain the bacteria.

***Note: Very few pus cells may be present if the patient has recently passed urine. Allow 2–4 hours after urination before collecting a samples.***

i. When culture is indicated (see previous test),
ii. Collect a sample of pus on a sterile cotton-wool swab.
iii. If possible, before inserting the swab in a container of Amies transport medium, inoculate a plate of culture medium.
iv. Label the samples and deliver them to the laboratory as soon as possible.
v. Isolation of *N. gonorrhoeae* from urine

***Note*:**

- A rectal swab is also required from homosexual patients. A selective medium is required to isolate *N. gonorrhoea* from a rectal sample.
- In acute urethritis, it is often possible to detect *N. gonorrhoea* in pus cells passed in urine, especially the first voided urine of the day (centrifuged to sediment the pus cells).

### *1.5.4 Cervical* swabs and possible pathogens

**A.** ***From non-puerperal women:***

*Neisseria gonorrhoea, Chlamydia trachomatis* (serovars D-K), *Streptococcus pyogenes*, herpes simplex virus.

**B.** ***From women with puerperal sepsis or septic abortion:***

*Streptococcus pyogenes*, other *beta*-haemolytic streptococci, *Staphylococcus aureus, Enterococcus* species, anaerobic cocci, *Clostridium perfringens, Bacteroides, Proteus, Escherichia coli and other coliforms, Listeria monocytogenes.*

### *1.5.5 Collection of cervical samples from female patients*

i. A samples collected from the endocervical canal is recommended for the isolation of *N. gonorrhoeae* by culture. Use a sterile vaginal speculum to examine the cervix and collect the samples.
ii. Moisten the speculum with sterile warm water, and insert it into the vagina.
iii. Cleanse the cervix using a swab moistened with sterile physiological saline.
iv. Pass a sterile cotton-wool swab 20–30 mm into the endocervical canal and gently rotate the swab against the endocervical wall to obtain a samples.
v. When gonorrhoea is suspected, before inserting the swab in Amies transport medium, if possible inoculate a plate of culture medium. Label

the sampless and deliver to the laboratory as soon as possible. Inoculated culture plates must be incubated within 30 minutes.

### *1.5.6 Vaginal Swabs*

Vaginal discharge may be due to infection of the vagina or infection of the cervix or uterus. Pathogens causing vaginal infections include *Trichomonas vaginalis, Candida species, and Gardnerella vaginalis with anaerobes.*

### *1.5.7 Collection* of vaginal discharge

To detect *T. vaginalis, C. albicans* and *G. vaginalis*

Two preparations are required:

**A. Wet preparation to detect motile *T. vaginalis***

i. Use a sterile swab to collect a samples from the vagina.
ii. Transfer a sample of the exudate to a microscope slide.
iii. Add a drop of physiological saline and mix.
iv. Cover with a cover glass.
v. Label and deliver to the laboratory for immediate examination

**B. Dry smear for Gram staining to detect Candida and examine for clue cells**

Although yeast cells can be seen in an unstained wet preparation, the Gram positive cells and pseudohyphae of *C. albicans* are more easily seen in a Gram stained smear.

i. Use a sterile swab to collect a samples from the vagina.
ii. Transfer a sample of the exudate to a microscope slide and spread it to make a *thin* smear.
iii. Allow the smear to air-dry, protected from insects and dust.
iv. Label and deliver to the laboratory with the wet preparation.

### *1.5.8 Collection of samples to detect T. pallidum*

To detect motile *T. pallidum* spirochetes, a samples must be collected before antibiotic treatment.

i. Wearing protective rubber gloves, cleanse around the ulcer (chancre) using a swab moistened with physiological saline. Remove any scab which may be present.
ii. Gently squeeze the lesion to obtain serous fluid. Collect a drop on a clean cover glass and invert it on a microscope slide.
iii. Immediately deliver the preparation to the laboratory for examination by darkfield microscopy

## 1.6 WOUND SAMPLES TO INCLUDE PUS, ABSCESS, TISSUE, ETC.

Indicated for primarily to diagnose skin and soft tissue infections (SSTIs). SSTIs may be caused by a variety of organisms; different organisms depending on how the wound or injury occurred. Fungi, parasites, and viruses are also important causes of certain types of SSTIs.

Community-acquired: *Staphylococcus aureus, Streptococcus pyogenes, Clostridium perfringens* and other anaerobic bacteria).

Hospital-acquired: *Staphylococcus aureus,* Enteric Gram-negative rods – *E. coli, Pseudomonas aeruginosa, Acinetobacter* species, and other non-fermenting Gramnegative rods, *Streptococcus pyogenes, Clostridium* species and other anaerobic bacteria

### *1.5.9* Collection of Wound samples - *General considerations*

i. Samples should be collected by a medical officer or an experienced nurse.
ii. Pus from an abscess is best collected at the time the abscess is incised and drained, or after it has ruptured naturally.
iii. When collecting pus from abscesses, wounds, or other sites, avoid contaminating the sample with commensal organisms from the skin.
iv. As far as possible, a samples from a wound should be collected before an antiseptic dressing is applied.
v. When pus is not being discharged, use a sterile cotton-wool swab to collect a sample from the infected site.
vi. Immediately after collection, immerse the swab in Amies transport container.
vii. Label the samples and as soon as possible deliver it with a completed request form to the laboratory.
viii. ***When myeloma is suspected:*** Obtain a samples from a draining sinus tract using a sterile hypodermic needle to lift up the crusty surface over the sinus opening. This method of samples collection has the advantages that the pus obtained is usually free from secondary organisms and the draining granules can usually be seen clearly and removed for microscopic examination. Transfer the pus to a sterile container.

ix. ***When tuberculosis is suspected****:* Aspirate a sample of the pus and transfer it to a sterile container.

x. ***When the tissue is deeply ulcerated and necrotic*** (full of dead cells): Aspirate a sample of infected material from the side wall of the ulcer using a sterile needle and syringe. Transfer to a sterile container.

xi. ***Fluid from pustules, buboes, and blisters****:* Aspirate a samples using a sterile needle and syringe. Transfer to a sterile container.

xii. ***Serous fluid from skin ulcers, papilloma, or papules, that may contain Treponema****:* Collect a drop of the exudate directly on a *clean* cover glass and invert it on a *clean* slide. Immediately deliver the samples to the laboratory for examination by dark-field microscopy.

***Caution:*** Samples from patients with suspected plague or anthrax are highly infectious. Label such samples HIGH RISK and handle them with care. In a health centre for dispatch to a microbiology laboratory Collect the samples using a sterile cotton-wool swab.

Insert it in a container of Amies transport medium, breaking off the swab stick to allow the bottle top to be replaced tightly.

**In a hospital with a microbiology laboratory**

- Using a sterile technique, aspirate or collect from a drainage tube up to 5 ml of pus.
- Transfer to a leak-proof sterile container. When the material is aspirated fluid from a pustule, transfer the fluid to a sterile, leak-proof container. Stopper, and seal in a leak-proof plastic or metal container.

***Note: It is not possible to transport exudate from a suspected treponemal ulcer because the Treponema remain motile for only a short time.***

Make a smear of the material on a clean slide (for Gram staining) and allow to air-dry in a safe place. Heat-fix the smear.

***Caution:*** **Do *not* make a smear for transporting when the samples is from a patient with suspected anthrax.**

Send the samples with a completed request form to reach the microbiology laboratory within 6 hours.

## 1.8 COLLECTION OF THROAT AND NASAL SWABS

Throat cultures are performed to diagnose streptococcal pharyngitis (infection with *Streptococcus pyogenes* (Group A streptococci). The most common causes of pharyngitis (sore throat) are viruses, which cause over 75- 80% of all cases. Of the

bacteria that cause pharyngitis, Group A streptococci is the major cause and therefore, with few exceptions the only bacteria that is reported from a throat culture is *Streptococcus pyogenes.*

Exceptions include:

*Corynebacterium diphtheria* which in areas of the world where vaccination is prevalent, is a rare cause of pharyngitis

*Neisseria gonorrhoea* can cause pharyngitis, however many pharyngeal infections with *N. gonorrhoea* are mild or asymptomatic.

If the physician suspects either of these two organisms, he/she must let the lab know because the isolation of either requires special culture techniques.

### *1.5.10* Throat Swabs

Throat swab cultures are to be taken under direct vision with good light.

Areas of exudation, membrane formation, any inflammation or if not seen then tonsillar crypts are the sites of choice.

### *1.5.11* Nasal swabs

Nasopharyngeal swabs are better taken by treating physician/surgeon himself. For recovery of viral agents, washings are collected after gargles with nutrient broth by the patient.

Nasal Sample for Mycobacterium leprae

The nasal sample for *M. leprae* can be taken as follows:

**Nasal *swab***

i. Make the patient sit with his head bent backwards but facing the light.
ii. Insert and repeatedly rotate the swab into one of the nasal cavities, against upper part of the nasal septum.
iii. Make 2-3 evenly spread smears.
iv. Air dry the slides, wrap in a paper and send to the laboratory.

***Nasal washings and nasal blow***

i. Make the patient sit.
ii. Place a few drops of sterile saline in the nose.
iii. After 3 min, ask the patient to blow hard his nose on a small sheet of plastic or cellophane. (This plastic or cellophane can be given to the patient to take it home and ask him to blow hard onto the sheet, the following morning, soon after waking and before washing.
iv. The patient can bring it directly to the laboratory).
v. Transfer some of the mucus pieces from the washing to a slide with a clean wooden stick and make thin smear.

vi. Air dry slide and send it to the testing area

***Collection of Nasopharyngeal Swabs***

Assemble equipment for Nasopharyngeal swab collection and PPEs for prevention of infections.

i. Lable the VTM with required information and fill the register and all necessary forms with all necessary information.
ii. Remove the swab from the package. Do not touch the soft end with your hand or anything else.
iii. Insert the nasopharyngeal swab into the nasopharnx region.
iv. Leave in place for a few seconds.
v. Slowly remove swab while slightly rotating it.
vi. Break the applicator's stick end and put tip of swab into ATM containing vial VTM

**COLLECTION OF OROPHRYANGEAL SWABS**

Assemble equipment for orophryangeal throat swab collection and prevention of infections.

i. Label the VTM with required information and fill the register and all necessary forms with all necessary and correct information.
ii. Remove the swab from the package. Do not touch the soft end with your hand or anything else.
iii. Have the patient open his/her mouth wide.
iv. Insert swab in the area of tonsils.
v. Use tongue depressor if the patient is not able to resist gagging and closing the mouth while the swab touches the back of the throat near the tonsils.
vi. Rotate swab to obtain adequate sample
vii. Break the applicator's stick end and put tip of swab into vial containing VTM

## *1.6* COLLECTION OF SKIN SMEARS

i. Ensure all required material and supplier are in place.
ii. Ask the patient to sit on the prepared chair at phlebotomy.
iii. Confirm the identity of the patient by asking his/her name and compare with name written on the request form.
iv. Clearly explain to patient what you want to do and ask for verbal consent
v. Select the site, you should take a smear from two sites only**;**
vi. One ear lobe and One active lesion

### *1.6.1* Sample *from ear lobe*

i. Clean the skin at the smear site with swab and let it air dry. ii. Pinch the skin firmly between your thumb and forefinger
ii. Make an incision in the skin about 5mm long and 2mm deep. Keep on pinching to make sure the cut remains blood less iv. Turn the scalpel $90^{o}$ and hold it at right angle to the cut
iii. Scrap inside the cut once or twice with side of the scalpel to collect tissue fluid and pulp (there should be no blood in the sample as this may interfere with staining and reading of the slide
iv. Stop pinching the skin and absorb any bleed with dry cotton swab
v. Spread the material scrapped from the incision on to the slide, ensure you spread it evenly with the flat of the scalpel making 8mm diameter
vi. Clearly label your slide with patient ID similar to that appearing on patient request form

### *1.6.2* Sample *from active lesion*

i. Rub the scalpel with cotton wool drenched with alcohol.
ii. Pass the blade through the flame of the spirit burner for 3-4 seconds and let it to cool without touching anything
iii. Select the most active looking lesion (active means lesion that are raised and reddish in colour)

## 1.7 SPUTUM SAMPLES

Sputum cultures are performed to diagnose infections such as pneumonia and pulmonary tuberculosis that is caused by *Mycobacterium tuberculosis*. Bacteria associated with Community-Acquired Pneumonia (CAP):

i. *Streptococcus pneumoniae* – most common bacterial cause of CAP
ii. *Haemophilus influenzae*
iii. *Moraxella catarrhalis*
iv. *Staphylococcus aureus* - particularly following a viral infection such as influenza
v. *Klebsiella pneumoniae* – particularly in individuals with chronic conditions such as alcoholism
vi. *Mycoplasma pneumonia** – particularly in young individuals in closed quarters
vii. Bacteria associated with Hospital-Acquired Pneumonia:
    a. *Streptococcus pneumoniae*
    b. Enteric Gram-negative rods such as *E. coli, Klebsiella, Enterobacter, Citrobacter,* and *Serratia*
    c. *Staphylococcus aureus*
    d. *Pseudomonas aeruginosa*
    e. *Acinetobacter* species
    f. *Haemophilus influenzae*

### *1.6.3* Sputum *Collection Procedure*

i. Label the sample container on the body of the container, not on the lid and fill out the sputum examination request form.
ii. Instruct the patient and demonstrate how she or he can produce and collect good sputum;
    a. Access to a well ventilated place (outside the laboratory working area),
    b. Mouth wash (rinsing with water),
    c. Breathe in deeply 2-3 times, and breathe out hard each time.
    d. Cough deeply from the chest and collect the sputum into the container.
    e. Opening and closing the sputum container so as there are no leaks or smearing on the exterior of the container.
    f. Hand wash steps
iii. Emphasize the need for the patient to supply the most useful sample, the normally thick, yellowish (sometimes blood-streaked), purulent material brought up from the lungs after a deep, productive cough.
iv. Emphasize that saliva produced by spitting is not sputum. However, if the only sample the patient can produce is salivary, do submit it to the laboratory as it can still yield useful information.
v. Encourage the patient to bring the collected sample back to the unit as quickly as possible.
vi. For *M. tuberculosis* culture, a series of three fresh, early morning samples (5-10 ml) are collected and kept in the refrigerator. If amount is less, the patient is advised to collect 24 h sputum or until 50 ml is obtained.
vii. M.tuberculosis can be recovered from the gastric contents in infants, debilitated patients and those who are unable to cooperate in the collection of sputum. This can be obtained by gastric aspiration performed as an indoor procedure.
viii. Gastric washings are better collected early in the morning, in fasting state. These are neutralised soon after collection by N/10 NaOH.

# CHAPTER TWO: PARASITOLOGY

## 2.1. PROCEDURE FOR MALARIA RAPID TEST (MRDT)

### 2.1.1 Purpose

This procedure provides instructions for In vitro qualitative screening test for detection of malaria parasites (*P. falciparum, P. vivax, P. ovale and P. malariae*) in whole blood

### 2.2.2 Scope

This procedure applies to all Health Laboratory Practitioners who works in parasitology section on performing MRDT test.

#### *2.2.2.1 Responsible*

Head of section is responsible to ensure implementation and competence assessment for all staff that will perform this test

### 2.2.3 Principle

Test is based on principle of immune-chromatography in which nitrocellulose membrane is pre-coated with two monoclonal antibodies as two separates lines. One monoclonal antibody (test line PAN), is PAN specific to lactose Dehydrogenase (phLDH) of the plasmodium species (*plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*). And the other line (test line pf) consist of monoclonal antibody specific to histidine rich protein 2 (HRP2) of plasmodium falciparum. When the test sample along with assay diluent flows through intercellular membrane, monoclonal antibody conjugated with colloidal gold which are PAN specific to plDH and falciparum specific for HRP2 binds to plasmodium antigen released from lysed blood sample. These antigen –conjugate complex moves through the nitrocellulose membrane and binds to corresponding immobilised antibody at test lines, which leads to the formation of colour band/bands indicating reactive results. The control band will appear irrespective of reactive or non-reactive sample.

### 2.2.4 Sample Requirements

Whole blood from EDTA/Finger prick

### 2.2.5 Equipment

Timer

### 2.2.6 Materials

Gloves, Test kit, Lab Coat, and

### 2.2.7 Storage and Stability

MRDT sample should be tested within 1hour after collection if not possible stored at 2-8$^{o}$c for 7 days.

### 2.2.8 Safety

i. All personal protective equipment (PPE) must be worn when performing this procedure.
ii. All samples must be regarded as potentially infectious.
iii. Refer to National infection prevention and control Guidelines (IPC)

### 2.2.9 Calibration

Not Applicable

### 2.2.10 Quality Control

Process known positive and negative blood sample daily before performing patient samples.

### 2.2.11 Procedure Steps

Follow the actions described below for specimen collection step-by-step.

i. Allow all kit components and specimen to room temperature prior to testing
ii. Remove the test device from the foil pouch; place it on a flat, dry surface.
iii. Label the test device with the patient identification number/name
iv. Transfer 5µl of whole blood collected in an inverted cup/special capillary provided into sample well by touching sample pad. (see manufacture instruction)
v. Add 4 drops of assay diluents into the squire assay diluents well. (See manufacture instruction)
vi. Read the result within 15 min. (Do not interpret after 30 min) and read result. (See manufacture instruction).

### 2.2.12 Biological Reference Intervals

Not Applicable

### 2.2.13 Interpretation and Reporting of Results

**Negative**

i. The presence of one colour band (“C” Control line) within the result window indicates a negative result. **Positive**
ii. “C” and “*P. falciparum* the presence of two coloured bands (“Pf” test line and “C” control line) within the result window no matter which band appears first, indicate P.F positive result**. Positive**

iii. “C”, “P.f” and “Pan” the presence of three colored band (“Pf”, Pan “Test line and “C” Control line) within the result window no matter which band appears first, indicate P.F positive or mixed infection of P.F and P.V or P.M or P.O **Invalid result**
iv. If no coloured band appear, at control line “c” within stipulated time then the result is invalid.

### 2.2.14 Limitations of the procedure and sources of error

Test kit cannot detect malaria antigen if parasites are less than 100. The test is limited to detect HRP2, an antigen to Malaria Plasmodium species that may persist after treatment or passed P. falciparum infection.

### 2.2.15 Performance Characteristics

Refer to package insert

### 2.2.16 Supporting Documents

Sample collection manual

### 2.2.17 References

Manufacturer inserts

## 2.2. PROCEDURE FOR MALARIA MICROSCOPY

### 2.2.1 Purpose

This procedure provides instructions for the examination of malaria parasite to diagnose and monitor treatment outcome of malarial infection.

### 2.2.2 Scope

This procedure is used during examination of malaria parasites at the Laboratory using Microscopy.

### 2.2.3 Responsible

- Qualified and trained health Laboratory Practitioners are responsible for performing this test procedure
- The Head of Parasitology unit is responsible for ensuring the effective implementation and maintenance of this procedure.

### 2.2.4 Principle

#### *2.2.4.1 Giemsa stain*

Giemsa stain is a *Romanowsky* stain contains methylene blue which is basic stain and eosin is acidic stain, the malaria parasites has DNA in the nucleus which is basic in nature and RNA in the cytoplasm is acidic in nature, during staining the reactions takes place whereby acidic part of a parasite will pick-up basic part of the stain and the basic part of the parasite will pick-up the acidic part of the stain, that's why the nucleus of the parasite will show red color and the cytoplasm will stain bluish. Malaria parasites are identified by microscopic examination of thick or thin blood films stained with Giemsa. Thick blood films are used for detecting parasites, thin blood films are used for more detailed morphological examination and for determining parasite species. Thick blood films consist of several layers of blood cells, so that a large volume of blood is examined. The thick film staining technique ruptures red cells, leaving white cells and parasites intact. The thin film staining technique preserves the morphology of blood cells and malaria parasites.

### 2.2.5 Sample Requirements

Whole blood collected in EDTA tube is required Blood slides

### 2.2.6 Equipment

Hot plate, Tally counters or differential counter, Microscope, Timer, Weighing scale, and PH Meter

### 2.2.7 Maintenance

To increase the life-span of microscopes, preventive maintenance, including cleaning the objectives and replacing parts as necessary. should be part of routine internal QC and must be properly done and documented. Microscopes should be covered when not in use to avoid exposure to dust, and proper precautions must be taken in humid areas to avoid fungal growth on the lenses and in the microscope. Also Hot plate, Tally counter, Timer, weighing scale and PH meter should be maintained as per manufacture instructions.

### 2.2.8 Materials

- High-quality immersion oil should be used according to the manufacturer's recommendations.
- High-quality microscope slides, free of surface abrasions, preferably have a frosted end for labeling and purchased from a reputable supplier should be used.
- 10% alcohol-based Giemsa stain,
- markers, lancets, syringes, needles, Vacutainer-type needles, alcohol swabs, lens-cleaning solution, lens-cleaning tissues, buffer tablets, pH calibration solutions, cotton-wool, gloves, safety glasses (including the over-spectacle type), filter paper and glycerol. gloves, sharps, boxes, gowns and detergents, and slide boxes.

### 2.2.9 Storage and Stability

- Unstained blood slides should be stored at 2-8 c for 3 months
- Stained blood slides should be stored at room temperature for 3 months on slide boxes.

### 2.2.10 Safety

- Adhere to safety precaution as stated in safety manual/IPC guideline
- All personal protective equipment(PPE) should be worn when performing procedure All samples should be regarded as potential infectious

### 2.2.10 Calibration

Calibration of PH meter and weighing scale should be done once per year. Maintenance of microscopes should be done as planned.

### 2.2.11 Quality Control

QC slides should be used to check the quality and performance of the Giemsa stain, Microscope and Laboratory personnel. Malaria-positive and negative blood should be used to prepare QC thick and thin films. Before examining patient slides, the QC slides should be checked first, If the QC slides are satisfactory, the patient slides can be examined. Slides must be selected regularly for cross-checking, either by sending them to a crosschecking center or during routine supportive supervisory visits.

### 2.2.12 Procedure Steps

#### A. Preparation of thick and thin blood film

i. Prepare both thick and thin film on the same microscope glass slide.
ii. Label the slides with the unique patient ID including the date of examination.
iii. Put Slide card for making thin and thick blood films, showing size of blood drops and area of slide to cover for a thin film and thick film
iv. Put the microscope glass slide on the slide card for thick and thin film
v. Pipette 2µl of blood and pour it on the smallest circle on the slide card
vi. Place the spreader in front of the 2µl drop of blood at a $30^{o}$- $45^{o}$ angle. Use a clean microscope slide with a smooth edge as a spreader.
vii. Pull back the spreader and hold until the blood evenly spreads along the width
viii. Push the slide forward in a smooth continuous motion
ix. Avoid hesitation or a jerky motion when spreading the blood
x. Pipette 6µl of blood and pour on the large circle on the slide card.
xi. Using another slide, spread the drop of blood within the confined area to make a thick film.
xii. Put the slide films on the slide rack and allow it to dry.
xiii. Fix the thin smear for one second by either spraying or dipping into absolute methanol.
xiv. Air dry the slides on a slide rack with the fixed thin film facing down

#### B. Preparation of Giemsa Stock Solution (500ml) from Giemsa powder

i. Measure and dissolve 3.8g of Giemsa powder in 250ml of methanol
ii. Measure 250ml of glycerol and add to the solution above
iii. Ripen the stock solution by placing in direct sunlight for about 1 week or place in water bath of temperature 56$^{o}$C and shake at interval.
iv. Filter the stock solution before storing in a cool dry place with label and date of preparation

**C. Preparation of 10% Giemsa stain from Giemsa Stock solution**

i. Add 1 part of stock solution to the 9 parts of 7.2 buffer solution
ii. Prepare 30ml of 10% working giemsa solution as follows:
iii. 3mls of Giemsa +25ml of buffered water PH of 7.2, mix and transfer to a clean caped leakproof bottle
iv. Lable and keep in a dry place

**D. Staining of blood films**

i. Arrange the slides on a staining rack with the sample side facing up
ii. With an aid of a disposable pipette, flood the films with 10% Giemsa solution and leave to stain for 15 minutes.
iii. Decant the Giemsa and wash in buffered water at pH 7.2
iv. Clean the back of each slide with cotton wool or gauze
v. Air dry the slides

### 2.2.13 Examination of the blood films

i. Place a drop of immersion on the thick film
ii. Place the slide on the microscope stage
iii. Swing the X10 objective into position and bring the film into focus using the course adjustment**.**
iv. Use the X10 and X40 objectives to check quality of the slide(s) before reading and reporting;
v. Swing the X100 oil immersion objective into position and focus using the fine adjustment
vi. Choose the correct smear reading pattern either Horizontal, start from up right to left or Vertical, start from upright down.
vii. Systematically, Examine the thick film.
viii. If you do not see any parasites, continue examining the whole film.
ix. If parasites are seen, start counting number of white blood cells and parasites simultaneously up to a WBC of 200.
x. If the parasitemia level is less than 10/200WBC, continue to count up 500 WBC
xi. Report the number of parasites count per 200 or 500 white blood cells in the Blood parasites worksheet.
xii. Retain the read slides for 3 Months

### 2.2.14 Biological Reference Intervals Not Applicable

### 2.2.15 Reporting and Interpretation of Results Malaria parasites

- Typical Malaria parasites have the following features on the Giemsa stained films;
- Purple red chromatin dot, Blue cytoplasm, Brown-black/yellowish green pigment and Distinct morphology

#### *2.2.15.1 Interpretation*

In stained blood films, trophozoites appear as red stained chromatin dots with blue staining cytoplasm. If doubtful on parasites seen, search for definite ones. Do not make a diagnosis on the basis of structures that resemble rings or chromatin dots alone. Structures that may be confused with malaria parasites are platelets, portions or other red cell inclusion bodies

#### *2.2.15.2 Reporting*

If no parasites are seen report as “No parasites seen”

If malaria parasites are seen, report species identified and count e.g. “*Plasmodium species* seen 50/200WBC”

### 2.2.16 Critical values

The following are the Panic/Critical values for Malaria microscope test results. In case of the patient result falling inside the indicated values, call the Doctor or relevant ward and record the details of the conversation on the Panic Result Book

| **Test** | **Critical value** |
|---|---|
| Malaria for Under 5 years | >100 *P. falciparum* Asexual/200wbc’s |
| Malaria for >5 years& adult | >1000 *P. falciparum* Asexual/200wbc’s |
| None Tropical people | ANY POSITIVE |

### 2.2.17 Limitation of the Procedure and Sources of Errors

- Poor storage of reagents or using the reagents after expiry date may cause false results.
- Difficulty in distinguishing young ring-stage parasites

### 2.2.18 Perfomance characteristics

Refer to the method verification report of this procedure.

### 2.2.19 Supporting Documents Not Applicable

### 2.2.20 References


- Practical Laboratory Manual-Jane Carter and Orgenes Lema
- Standard Operating Procedure Essential Laboratory Tests {AMREF-2008} EXT 120
- Basic Malaria Microscopy Part I. Learner's Guide, Second edition, WHO.


## 2.3 PROCEDURE FOR URINE MICROSCOPY

### *2.3.1 Purpose*

The purpose of this procedure is to provide step by step instructions for performing macroscopic and microscopic examination of urine sediment samples

### *2.3.2 Scope*

This procedure is used during examination of urine samples in Parasitology section in medical laboratory.

### *2.3.3 Responsible*

The Registered medical laboratory personnel is responsible for effective implementation and maintenance of this procedure.

### *2.3.4 Principle*

#### *2.3.4.1 Macroscopic Examination*

The urine is visualized with naked eyes to determine its appearance (turbidity and colour).

#### *2.3.4.2 Microscopic Examination*

The urine sediment is analyzed by a microscope to observe the presence of white blood cells, red blood cells, parasites and other abnormalities in urine sample. Identification of cells (WBC (pus cells), RBGs and Epithelial), casts, crystals, amorphous phosphates, bacteria and parasites in urine is based on their different cellular and intra-cellular morphology under light microscopy.

### *2.3.5 Sample Requirements*

20 mL, minimum 1 mL Fresh, cleanly voided urine collected in a clean container

Early morning voided mid-stream urine. Other samples include;

Random urine (collected at any time of the day)

Terminal urine sample collected at any time of the day for demonstrating ova of *Schistosoma haematobium*

First voided urine sample in the morning is used to demonstrate *Trichomonas vaginalis* in males

In infants and babies, a random urine sample collected as early as possible is used for all types of urine investigation.

### *2.3.6 Equipment*

Microscope, Centrifuge machine, Refrigerator, Timer, and Forceps

### *2.3.7 Materials*

Gloves, Cover slips, Glass slides, Test tubes, Centrifuge tubes, Gauze, Grease pencil, Lens paper, Waste containers, Urine container, Laboratory coat, Marker pen

### *2.3.8 Storage and stability*

Process urine sample within 1hour of collection, if not possible refrigerate at 2-8$^{o}$C immediately and test within 12 hours.

### *2.3.9 Safety*

i. Adhere to safety precautions as stated in the Safety Manual
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.
iv. Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### *2.3.10 Calibration*

Urine analyzers, timer and centrifuge should be calibrated as per schedule Maintenance of all equipments should be done as planed

### *2.3.11 Quality Control*

Quality control should be performed daily before processing patient samples by analysing known positive and negative samples.

### *2.3.12 Procedure*

#### *2.3.12.1 Macroscopic examination*

Observe the appearance of the urine for color and clarity/turbidity

### *2.3.12.2 Microscopic examination*

i. Label the centrifuge tubes with the laboratory unit number using a grease pencil and arrange into a rack.
ii. If the urine sample is to be tested both for urinalysis and culture, mix well by inverting three times and aliquot the sample into a centrifuge tube and label exactly as the original sample
iii. Transfer the centrifuge tubes containing urine sample into the centrifuge.
iv. Make sure the centrifuge tubes are balanced well.
ii. Centrifuge the urines at 3000rpm for 5 minutes.
iii. Remove the centrifuge tubes from the centrifuge
iv. Pour off the supernatant into the sink with running water and leave the urine deposit
v. Transfer the rack containing arranged centrifuge test tubes to the working area
vi. Arrange the marked slides according to the number of urine sample
vii. Re-suspend the urine deposit by tapping the bottom of the centrifuge tube.
viii. Transfer a drop of the deposit onto a clean glass slide.
ix. Put on a cover slip
x. Place the slide on the microscope stage and reduce the iris diaphragm
xi. Examine the slide systematically using x 10 objective and count the cells, casts, crystals, amorphous phosphates and parasites (these structures are more likely to be seen around the edges of the cover slip.
xii. Swing the x40 objective into position
xiii. Open the iris diaphragm very slightly to allow just enough light to provide a contrast of cells, casts, crystal and amorphous phosphates against the bright back ground (these structures are more likely to be seen around the edges of the cover slip).
xiv. structures cannot be seen if a very bright light is used
xv. Put the used glass slide and cover slip in a container with disinfectant.

### *2.3.13 Biological Reference interval*

**Macroscopy**

| | |
|---|---|
| Color | Straw - Dark yellow |
| Appearance | Clear - Hazy |

***Microscopy***

| | |
|---|---|
| Red blood cells: | 0–2/hpf. |
| WBC (PUS CELL)/Pus cells: | 0–5/hpf |
| Casts | 0-4/hpf |
| Bacteria | Negative |

### *2.3.14 Interpretation and Reporting of Results*

**A. Macroscopic**

Report the Turbidity and color of the urine e.g. Clear yellowish, Blood stained etc.

**B. Microscopic**

- Report a range of the actual highest number of WBC (pus cells), RBC and Parasites per high power field as counted under x40 objective (e.g. 0 – 2 RBC/HPF, 3 – 5 WBC/HPF, 10 – 12 S. haematobium ova/HPF) etc.
- For crystals, Epithelial cells, yeasts or casts, report as +, ++ or +++.
    - Where + indicates 1 to 9 observed findings per field;
    - ++ indicates 10 to 100 observed findings per field;
    - +++ indicates above 100 observed findings per field.
- If no cells or parasites are seen report as “No parasites or cells seen.”

C. **Results interpretation**

- *Schistosomes Haematobium* ova in urine indicates Schistosomiasis disease
- Presences of more than 3 Red blood cells for males, more than 8 red blood cells for females and any RBC or WBC (pus cell) for children and notification of more than 8 WBC (Pus Cell) in either male or female is pathological significant.
- White blood cells may indicate infection or inflammation.
- Red blood cells may indicate kidney disease, a blood disorder, or bladder cancer.
- Bacteria can indicate infection.
- Skin cells can indicate infection or kidney disease.
- Crystals may be a sign of kidney stones.
- Casts, or tube-shaped proteins, may be a sign of a kidney disorder.
- Parasites can indicate parasitic disease in various parts of the body.

### *2.3.15 Limitations of the Procedure and Sources of Errors*

- Inadequate centrifugation of sample
- Poor collection of urine sample
- Prolonged storage of urine sample at wrong temperature Expired reagents
- Technical competency level
- Presence of artifacts such as plant cells

### *2.3.16 Performance Characteristics Not Applicable*

### *2.3.17 Supporting document Sample Collection Manual*

### *2.3.18 References*


- Monica Cheesbrough: District Laboratory Practice in Tropical Countries, Vol 1, Tropical Health Technology, 1998.
- Practical Laboratory Manual-Jane Carter and Orgenes Lema

## 2.4 PROCEDURE FOR STOOL ROUTINE EXAMINATION

### 2.4.1 Purpose

This procedure provides instructions for examination of stool macroscopic and microscopic to detect abnomalities/Parasites. The most common parasites include the roundworms *Ascaris lumbricoides* and *Necator americanus* (commonly called hookworm); the tapeworms *D. latum*, *Taenia saginata*, and, rarely, *T. solium*; the amoeba *E. histolytica*; and the flagellate *G. lamblia*.

### 2.4.2 Scope

This procedure is used during examination of stool in Parasitology Section.

#### 2.4.2.1 Responsible

- The Registered medical laboratory personnel are responsible for implementing this test procedure.
- The Head of Parasitology section is responsible for ensuring the effective implementation and maintenance of this procedure

### 2.4.3 Principle

Normal saline retains the morphology of the organism in its natural shape and color, free helminthes eggs from debris.

Iodine - stains the internal structure of cyst to brown/yellow color so to allow the study of cyst morphology. It also kills the organisms to allow internal structure easily seen.

### 2.4.4 Sample Requirements

Stool collected in clean plastic screw capped container which has spoon like. Include macroscopic worms or worm segments as well as bloody and mucoid portions of the sample.

About 150mg stool should be collected.

### 2.4.5 Equipment

Microscope, Tally counters or differential counter, Centrifuge and Fume hood

#### *2.4.5.1 Maintenance*

Maintenance of microscopes should be done as planned

#### *2.4.5.2 Materials*

Normal saline, 1% Iodine, 10% formalin, Wooden applicator stick, Grease pencil, Gloves, Microscopic slides, Cover slip, Marker and Stool container

#### *2.4.5.3 Storage and stability*

Stool sample should be at the laboratory within two hours after collection. If a liquid or soft stool sample can't be examined within 30 minutes of passage, place it in a preservative; if a formed stool sample can't be examined immediately, refrigerate it or place it in preservative.

### 2.4.6 Safety

- All samples must be considered as potentially infectious and must be handled and examined with care.
- All personal protective equipments (PPE) should be worn when performing procedure
- Adhere to safety precautions as stated in the Safety manual
- Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### 2.4.7 Calibration

Calibration of centrifuge should be done as per schedule

### 2.4.8 Quality Control

Quality control should be performed daily before processing patient samples by using known positive and negative samples

#### *2.4.8.1 Procedure*

**A.Macroscopic Observations**

Observe the stool sample for the following; Color of the samples, Consistency, Presence of blood, mucus, and, or, pus. Whether the samples contain worms.

**B.Wet Preparation by Saline and 1% Iodine**

i. Using marker label a microscope slide with the laboratory number
ii. Place a drop of fresh physiological saline at one end of a slide and a drop of 1% Iodine on the other end.
iii. Using applicator stick, pick up a size of match head stool (~2 mg) and mix with a drop of saline and a similar amount with Iodine to make a smooth thin preparation.

iv. If stool is formed, the portion should include the inside and outside parts of the sample

iv. For mucoid or watery stool, mix the entire contents before picking a portion to mix with saline.

v. Cover the preparations with a cover slip.

vi. Start with 10x objective to systematically, examine the entire saline preparation.

vii. Use the 40x objective to assist in the detection and identification of parasitic elements (eggs, ova, cyst etc). Always examine several microscope fields with this objective before reporting 'No parasites seen'.

viii. Use the iodine preparation to assist in the identification of cysts.

ix. Report the findings on Stool analysis worksheet.

x. Discard the used microscope slide on the container with disinfectant.

### 2.4.9 Biological Reference Intervals

Not Applicable

### 2.4.10 Interpretation and Reporting of Results

#### *2.4.10.1 Interpretation*

i. Adult helminths or portions of helminths may be recovered and seen with a naked eye. Examples include *E. vermucularis* adult worms, Ascaris lumbricoides adult worms, and tapeworm proglottids.

ii. Occasionally, other helminths may be recovered (hookworm, Strongyloides stercoralis), but identification requires the use of the microscope.

iii. The appearance of stool will yield diferrent interpretation such as; blood and mucus in faeces: might be suggestive of amoebic dysentery, intestinal schistosomiasis, invasive balantidiasis (rare infection), and severe *T. trichiura* infections. Other non-parasitic conditions in which blood and mucus may be found include bacillary dysentery, *Campylobacter enteritis*, ulcerative colitis, intestinal tumour, and haemorrhoids.

iv. **Presence of pus**: This can be found when there is inflammation of the intestinal tract. Many pus cells can be found in faecal sampless from patients with bacillary dysentery. They can also be found in amoebic dysentery but are less numerous.

v. **Pale coloured and frothy (containing fat) samples:** might be suggestive of giardiasis and other infections as- sociated with intestinal malabsorption.

vi. **Pale coloured faeces:** (lacking stercobilinogen) might be suggestive of an obstructive jaundice.

vii. Mucoid and blood diarrhea might be suggestive to presence of *E. histolytica*

#### *2.4.10.1 Reporting*

**A.Macroscopic Findings - Report the following:**

i. Colour of the samples.

ii. Consistency, i.e. whether formed, semiformed, unformed, watery.

iii. Presence of blood, mucus, and, or, pus. If blood is present note whether this is mixed in the faeces. If only on the surface this indicates rectal or anal bleeding.

iv. Presence of worms, e.g. *A. lumbricoides* (large roundworm), *E. vermicularis* (threadworm) or tapeworm segments, e.g. *T. solium*, *T. saginata.*

**B. Microscopic Findings – Report the Following**

- Report presence of any ova, trophozoites or cysts seen, specifying the species, e.g. "*Entamoeba histolytica* trophozoites seen".
- If no parasites are seen report as " No ova or cysts seen".

### *2.4.10.2 Limitation of the Procedure and Sources of Error*

- Delay in examination of stool sample may cause missing of some parasites in wet prepation which are dectected still alive. Examples of such organisms are Strongloides stercolaris, Giardia lamblia *E. Histolytica* trophozoites, etc.
- Presence of urine kills trophozoites (false-negative results). Excessive heat or cold.

### *2.4.10.3 Performance Characteristics*

Refer to the method verification of this procedure.

### *2.4.10.4 Supporting Documents Not Applicable*

### *2.4.10.5 References*


- Practical Laboratory Manual-Jane Carter and Orgenes Lema
- Monica Cheesbrough: District Laboratory Practice in Tropical Countries,Vol 1,
- Tropical Health Technology, 1998.
- Brunner & Suddarth's Handbook of Laboratory and Diagnostic Tests, 2010.


## 2.5 PROCEDURE FOR EXAMINATION OF BLOOD FOR MICROFILARIAE

### 2.5.1 Purpose

This procedure provides instructions for processing of blood, lymphatic, and cerebrospinal fluid for the recovery of lymphatic filariasis. Diagnosis of filarial infections is often confirmed by demonstration of the parasite.

### 2.5.2 Scope

This procedure is used during examination procedures to diagnose filarial infections in the Laboratory using Microscopy.

### 2.5.3 Responsible

- Qualified and trained Medical Laboratory Technician, s are responsible for performing this test procedure.
- The Head of Parasitology unit is responsible for ensuring the effective implementation and maintenance of this procedure.

### 2.5.4 Principle

#### A. Giemsa stain

Giemsa stain is a Romanowsky stain contains methylene blue which is basic stain and eosin is acidic stain, the parasites has DNA in the nucleus which is basic in nature and RNA in the cytoplasm is acidic in nature, during staining the reactions takes place whereby acidic part of a parasite will pick-up basic part of the stain and the basic part of the parasite will pick-up the acid part of the stain, that's why the nucleus of the parasite will show red color and the cytoplasm will stain bluish.

#### B. Wet preparation

Microfilariae are seen in wet preparations of blood on direct microscopic examination or in the deposit of a blood sample after lysis with formalin and centrifugation.

### 2.5.5 Sample Requirements

Whole blood collected in EDTA tube should be collected around midnight (22:00 – 04:00 for *W. Bancrofti* and 10:00 – 15:00 for *L. Loa*), as this is the time when parasite is present in the blood for microfilaria worms.

### 2.5.6 Equipment

Hot plate, Tally counters or differential counter, Microscope, Centrifuge, Timer, Weighing scale and pH Meter

### 2.5.7 Materials

- 10 % Giemsa stain working solution,
- 70% Methylated spirit,
- Distilled water,
- Immersion oil,
- Tap water,

- buffer solution,
- Cover slips,
- Glass slide,
- Rack,
- Slide drying rack,

### 2.5.8 Storage and Stability

The Giemsa stock stain should be stored in a dark bottle and take precautions to avoid moisture entering the stain.

### 2.5.9 Safety

- All sampless must be considered as potentially infectious and must be handled and examined with care.
- All personal protective equipments (PPE) should be worn when performing procedure
- Adhere to safety precautions as stated in the Safety manual
- Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### 2.5.10 Calibration

Calibration of centrifuge, pH meter and weighing scale should be performed as planned. maintenance of microscopes should be done as planned

### 2.5.11 Quality Control

Positive and negative control samples are processed once a day in the morning before patient samples.

### 2.5.12 Procedure Steps

**Step 1: Procedural Steps - Wet Preparation**

i. Collect 10 ml of venous blood and dispense it into 10 ml of water.
ii. Mix the blood gently in water and leave for 10 minutes to give time for all the red cells to lyze.
iii. Centrifuge the haemolyzed sample for 10 minutes at slow to medium speed, i.e. RCF 300–500 g.
iv. Using a Pasteur pipette, immediately remove and discard the supernatant fluid.
v. Transfer the sediment to a slide, add a small drop of methylene blue and cover with a cover glass. The stain will be taken up by the nuclei and show whether the microfilariae are sheathed.

vi. Examine the entire preparation microscopically for motile microfilariae using the 10× objective with the condenser iris closed sufficiently to give good contrast.
vii. Count the number of microfilariae in the entire preparation. Divide the number counted by 10 to give the approximate number of microfilariae per ml of blood (mf/ml).
viii. If unable to identify the species with certainty, continue with step 2 (examination with 10% Giemsa stain under oil immersion objective).

**Step 2: Procedural Steps – 10 % Giemsa Staining Procedure**

i. Remove the cover glass and add a small drop of plasma, serum, or albumin solution.
ii. Mix and spread thinly. Allow the preparation to dry completely.
iii. The addition of albumin, plasma, or serum (known to be microfilaria-free) will help to prevent the preparation from being washed from the slide during staining.
iv. Fix with absolute methanol or ethanol for 2–3 minutes.
v. Flood the films with 10% Giemsa solution and leave to stain for 15 minutes.
vi. Decant the Giemsa and wash in buffered water at pH 7.2 or distilled water
vii. Clean the back of each slide with cotton wool or gauze
viii. Place a drop of immersion on the thick film and place the slide on the microscope stage
ix. Swing the x10 objective into position and bring the film into focus.
x. Swing the x100 oil immersion objective into position and focus.
xi. Examine the thick film systematically starting from the top left hand corner and move from field to field.

### 2.5.13 Biological Reference Intervals - Not Applicable

### 2.5.14 Reporting and Interpretation of Results

#### A. Microfilaria

Species are identified by noting the arrangement of the nuclei towards the end of the tail and the presence of sheath. *W. bancrofti* and Loa loa have a sheath; *M. perstans* does not have a sheath. In *Loa loa* and *M. perstans*, the nuclei reach the tail tip and the tail tip is rounded. In *W. bancrofti*, the tail tip is pointed.

#### B. Trypanosomes

Trypanosomes have elongated, flat, narrow bodies, often curved. In wet preparations, they move rapidly by means of an undulating membrane and flagellum. In stained preparations, the kinetoplast, a dark staining round body from which the flagellum originates, is seen.

### 2.5.15 Limitation of the Procedure and Sources of Errors

- Incorrectly timed samples for microfilaria or trypanosomes
- Poor storage of reagents or using the reagents after expiry date

### 2.5.16 Performance Characteristics

Refer to the method verification report of this procedure.

### 2.5.17 Supporting Documents Sample collection manual

### 2.5.18 References


- Practical Laboratory Manual-Jane Carter and Orgenes Lema
- Standard Operating Procedure Essential Laboratory Tests {AMREF-2008} EXT 120

# CHAPTER THREE: BLOOD TRANSFUSION

## 3.1 PROCEDURE FOR ABO AND RHESUS BLOOD GROUPING

### 3.1.1 Purpose

This procedure provides instructions for performing ABO and Rhesus (D) blood grouping using tube method.

### 3.1.2 Scope

This procedure is used in Blood Transfusion unit when performing ABO & Rhesus (D) blood group typing for donors and patients.

### 3.1.3 Responsible

The head of Blood Transfusion and competent medical laboratory personnel are responsible for ensuring this procedure is effectively implemented and maintained.

### 3.1.4 Principle

This method is based on immunophenotyping principle. The known antibodies A&B (antisera) react with unknown antigens on the red cells surface to form agglutination or haemolysis; this is known as **forward or cells grouping.** Also known antigens (A and B) react with unknown antibodies in the patient or donor serum/plasma to form agglutination or haemolysis; this is known as **backward or serum blood grouping.**

The D antigen on the red cells surface reacts which known D antibodies (anti-D) to form agglutination which determines the Rhesus group of an individual, either as Rh (D) **Positive** (agglutination) or Rh (D) Negative (no agglutination).

### 3.1.5 Sample Requirements

2-3mls of EDTA sample, 2-3mls of clotted sample from plain tube centrifuge the sample at 3500rpm (RCF) for 5 minutes.

### 3.1.6 Equipment

Centrifuge, Microscope, Refrigerator, Timers, 37˚C water bath and Thermometer

### 3.1.7 Materials

| Reagent | Consumables |
|---|---|
| Anti –A<br><br>Anti-B<br><br>Anti-D (saline, IgM)<br><br>Incomplete anti-D (IgG)<br><br>Anti-Human Globulin Serum (AGS)<br><br>0.85% Physiological Saline<br><br>Low ionic strength solution (LISS) | Gloves<br><br>Laboratory coat<br><br>Test tubes, Test tube rack, slides<br><br>Marker pens,<br><br>Beakers,<br><br>Pasteur pipettes, |

### 3.1.8 Storage and Stability

- Store serum or plasma at $2^0$C -$8^0$C for 7 days.
- Whole blood is stored at $2^0$C - $8^0$C for 3 days
- Anti-sera should be kept at $2^0$C – $8^0$C or as per manufacturer instructions

### 3.1.9 Safety

- Adhere to safety precautions as stated in the Safety manual/ IPC guideline
- All personnel protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### 3.1.10 Calibration

Calibration of auxiliary equipment should be done as per calibration schedule

### 3.1.11 Quality Controls

Perform Quality Controls once in a week, when new antisera received and when new control cells are prepared.

#### *3.1.11.1 Controlling of anti-sera*

- Commercial anti-sera are quality controlled by reacting them with known cell Suspension of A, B, O, Rh (D) Positive and Rh (D) Negative.
- Arrange 6 tubes labelled A, B, AB, O and D positive and D negative.
- Put one drop of 2-5% cell suspension of A, B, AB, O Rh (D) Positive and O Rh (D) Negative to the corresponding tubes above
- Add one drop Anti-A, Anti-B, Anti-AB and Anti-D into corresponding tubes.

### *3.1.11.2 Controlling of O sensitized cells*

- Label two tubes as O sensitized cells and O un sensitised cells
- Put one drop of corresponding of 3-5% cell suspension in the two tubes above.
- In the two tubes, add one drop of AHG each.

### *3.1.11.3 Controlling of physiological saline*

- Label two tubes as saline and distilled water
- Put one drop of saline and one drop of distilled water to the respective tubes above
- Add one drop of O Rh (D) Positive cells to the two tubes

### *3.1.11.4 Common step*

- Centrifuge all tubes above at light speed (1000rpm for 1 minute).
- Agglutination or haemolysis indicates positive reactions.
- Expected reactions are shown in table below
- Record results on ABO & Rh blood.

### *3.1.11.5 Interpretation of IQC results*

| **Commercial Antisera** | **Cell suspensions** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **A cells** | **B cell s** | **AB cells** | **Rh D pos cells** | **Rh D Neg Cells** | **O - sensitized cells** | **Unsensitised cells** |
| **Anti A** | + | - | + | - | | | |
| **Anti B** | - | + | + | - | | | |
| **Anti AB** | + | + | + | - | | | |
| **Anti D** | | | | | + | | |
| **LISS** | | | | | | +++ | |
| **AHG** | | | | | | + | - |
| **Saline** | | | | | | + to ++ | |
| **Distilled water** | | | | | | Haemolysis | |

**Key**:

+ Agglutination

- No agglutination

***Note: Strength of agglutination is graded from 1+ (separate agglutination) to 3+ (one solid agglutination)***

### 3.1.12 Procedure Steps

***Prepare of 2- 5% cell suspension***

i. Place 2-3 drops of donor red blood cells into a tube
ii. Fill the tube (3/4) with 0.85% normal saline
iii. Centrifuge the tube at 3400rpm for 2 to 3 minutes. Decant supernatant fluid. (Repeat 3 times)
iv. Transfer a drop of packed red cells from the above tubes and add 19 drops of saline to make 2% to 5% donor red cell suspension

### 3.1.13 Blood grouping procedure

**A.** ***Forward/Cell Grouping***

i. For each patient/donor label 3 test tubes as Anti-A tube (A), Anti-B tube **(B)**, and anti-D tube (D).
ii. Arrange the labelled test tubes in the test tube rack
iii. Add one volume of anti A into tube A
iv. Add one volume of anti-B into tube B
v. Add one volume of anti-D into tube D
vi. To each of the above tubes add one drop of 2 - 5% cell suspension and mix well.
vii. Centrifuge the three tubes at 1000rpm for one minute
viii. Examine the contents of the tubes for the evidence of agglutination.
ix. Read, interpret, and record the test results.

**B.** ***Backward/Serum Grouping***

i. For each patient/donor, label 2 test tubes as A cells and B cells.
ii. Add two drops of serum to each tube. iii. Add one drop of A reagent cells into tube labelled A cells,
iii. Add one drop of B reagent cells into tube labelled B cells
iv. Mix the contents of the tubes gently
v. Centrifuge the tubes at 1000 rpm for one minute.
vi. Examine the serum for evidence of haemolysis, gently suspend the cell bottoms and examine them for agglutination macroscopically and microscopically.
vii. Read, interpret and record test results.

#### *3.1.13.1 Procedure for Weak Rh (D)*

i. If the above anti-D reaction is negative, confirm weak Rh (D) as follows:
ii. Add two drops of LISS
iii. Incubate at 37˚C for 15 minutes in water bath. In absence of low ionic strength solution (LISS) incubate at room temperature for 30 minutes
iv. Spin for 1000rpm for 1 minute
v. Observe for agglutination macroscopically and microscopically or for haemolysis

vi. If agglutination or haemolysis is observed at this stage, report result as Rh (D) Positive and the procedure ends here.
vii. If there is still no agglutination, proceed as follows:

a. Wash contents of the tube 3 times with physiological saline
b. Discard supernatant after third wash
c. Add one drop Anti-Human Globulin Serum (AGS)
d. Spin for 1000rpm for one minute
e. Observe for agglutination (macroscopically and microscopically) or haemolysis
f. If agglutination is observed at this stage, report results as Rh (Du) Positive
g. If reaction is still negative, add one drop of O sensitised cells to the tube
h. Spin for 1000rpm for 1minute
i. Observe for agglutination (macroscopically and microscopically) or haemolysis
j. Presence of agglutination or haemolysis indicates a valid negative result.
k. Absence of agglutination or haemolysis means the test is invalid; therefore, it has to be repeated.

***Note: For purposes of transfusion, patients with Rh(Du) Positive should be given rhesus negative blood.***

### 3.1.14 Biological Reference Intervals

Not Applicable

### 3.1.15 Interpretation and Reporting of Results Interpretation of results

| **Patient/Donor Cell Grouping** | | | | | **Patient/ Donor Serum Grouping** | | |
|---|---|---|---|---|---|---|---|
| Anti-A | Anti-B | Ant-AB | Anti-D | Blood group & Rhesus factor | A-cells | B-cells | Blood group |
| + | - | + | + | A Rh (D) Pos | - | + | A |
| - | + | + | + | B Rh (D) Pos | + | - | B |
| + | + | + | + | AB Rh (D) Pos | - | - | AB |
| - | - | - | + | O Rh (D) Pos | + | + | O |

**KEY: + Means Agglutination**

**- Means No Agglutination**

#### *3.1.15.1 Reporting of results*

Results report should include the ABO Type and Rhesus D reaction results, e.g. Blood group A Rh (D) Positive, or Blood Group A Rh (D) Negative

### 3.1.16 Limitations of the Procedure and Source of Error

- Avoid haemolysed samples as this may lead to false negative results.
- Patients who have had recent multiple transfusions may develop alloantibodies that can interfere with antigen – antibody reactions

### 3.1.16 Performance Characteristics

Refer to the method verification report

### 3.1.17 Supporting Documents

Sample collection manual, safety manual, and quality manual.

### 3.1.18 References


i. Technical manual of the American Association of Blood Banks
ii. Mollison P.L., Blood Transfusion in Clinical Medicine 8th Ed. Oxford. Blackwell Scientific, Practical haematology by Decie iii. Guidance manual on "ABO and Rh blood grouping" (Institute of Biologicals-India)
iii. Anti-sera insert kit (Anti-A, Anti- B, Anti-AB, Anti- D Monoclonal blood grouping antibodies for tube and slide test) T Tulip diagnostics (P) LTD.


## 3.2 PROCEDURE FOR ESTIMATION OF HAEMOGLOBIN BY USING COPPER SULPHATE SOLUTION

### 3.2.1 Purpose

To provide guidance on the procedure of Haemoglobin estimation level to blood donors using the Copper Sulphate solution technique

### 3.2.2 Scope

This procedure provides guidance on haemoglobin estimation of blood donors using copper sulphate ($CUSO_4$) method in NBTS Blood collection teams and its satelites.

### 3.2.3 Responsible

Trained qualified and competent certified registered medical personnel and other authorised medical personnel.

### 3.2.4 Principle

A blood droplet is allowed to fall into copper sulphate solution of a specific gravity 1.053 and the movement of droplet is observed, If the specific gravity is higher than solution, the drop will sink within 15 sec or else it will remain suspended for some time.

### 3.2.5 Sample Requirements

Capillary Blood

### 3.2.6 Equipment

Not applicable

### 3.2.7 Materials

- **Reagents:** 70% Ethyl alcohol, Copper Sulphate solution with specific gravity of 1.053
- **Consumables:** Sterile swabs, Picker, Sharp container, Capillary tubes, Gloves, Universal bottle, Waste containers and Timer

### 3.2.8 Preparation of Copper II Sulphate solution

i. Weigh 170 gms of hydrous Copper Sulphate Powder/Crystals
ii. Put into volumetric flask
iii. Dissolve 170gms of hydrous Copper Sulphate Powder/Crystals with 1 litre of distilled water.
iv. Mix well until all Crystals dissolves
v. Label the solution as STOCK SOLUTION with Preparation date, Batch number and expiry date

*NB: Calculate expiry date six months from date of preparation*

vi. Store and keep stock solution at room temperature in a tightly capped brown glass bottle.

### 3.2.9 Prepare Working Solution

*To prepare 1 Litre* of *working solution;*

- Dispense 480mls of distilled water using measuring cylinder into a volumetric flask.
- Add 520mls of prepared stock solution using measuring cylinder - Mix thoroughly

### 3.2.10 Storage and Stability

Copper Sulphate working solution stored at room temperature in brown bottle in three months

### 3.2.11 Safety

i. Decontaminate working surfaces twice daily, in the morning and afternoon and when needed, all generated records are kept.
ii. Adhere to safety precautions as stated in the Safety manual
iii. All personal protective equipment (PPE) must be worn when performing this procedure.
iv. All samples must be regarded as potentially infections.
v. Refer to National infection prevention and control Guidelines for health waste management and safety practice.
vi. All spills should be wiped thoroughly using 0.5% sodium hypochlorite solution.

### 3.2.12 Calibration

Not applicable

### 3.2.13 Quality Control

**Reagent:** Test specific gravity of copper sulphate solution using Hydrometer

i. Accept the copper sulphate solution if the specific gravity is 1.053
ii. Reject copper sulphate if specific gravity is not equal to 1.053
iii. Record findings of the copper sulphate inspection and testing report form.
iv. Then select sample with slightly bellow and slightly high with a cut off value of High (12.6 -13.0) g/dl and Low (12.0 – 12.4) g/dl.
v. Accept the copper sulphate solution, if drop of blood with haemoglobin level less than 12.5 g/dl floats and blood with haemoglobin level higher than 12.5g/dl sinks. Perform corrective action if results are outside an acceptable limits

### 3.2.14 Procedure Steps

Follow the actions described below step-by-step:

i. Explain the Procedure to the Blood Donor
ii. Welcome and greet the donor, offer a chair to seat
iii. Explain the procedure and reassure the donor.
iv. Select and clean Site
v. Select donors' middle finger or the finger medial to the middle finger
vi. Clean the upper top of right-side area with cotton wool swab soaked in 70% ethyl alcohol in a spiral movement starting at the intended site out ward
vii. Leave it until dried do not blow the site
viii. Remove the lancets cover

ix. Using the index finger and thumb, squeeze and hold the donor's·fingertip at the upper joint tightly and prick
x. Wipe off the first drop of blood once with a dry swab
xi. Draw blood into the Capillary Tube
xii. Gently press the pricked fingertip to draw blood Note; Do not Squeeze the pricked finger because it may introduce tissue fluid, dilute blood and give false low Haemoglobin
xiii. Hold Capillary tube at approximately 60 degrees and let the blood flow into the capillary tube up to not less than 3/4 capacity
xiv. Avoid air entering the capillary tube by ensuring smooth flow and undisrupted flow of blood into the capillary tube
xv. Close the upper tip of capillary tube by placing your finger tip
xvi. Give clean dry swab to donor and instruct them to press finger with thumb till bleeding stops.
xvii. Release a drop of Blood to Copper Sulphate Solution
xviii. Hold capillary tube at least 1 cm above the surface of copper sulphate solution
xix. Release your finger tip to allow one drop of blood freely into labeled container of fresh daily prepared and quality control copper sulphate solution.

### 3.2.15 Biological Reference Intervals

Above or equal 12.5 g/dl

### 3.2.16 Interpretation and Reporting of Results

- Accept if HB is greater/equal than 12.5g/dl
- Reject if HB is less than 12.5 g/dl

### 3.2.17 Limitations of The Procedure and Sources of Error

Not applicable

### 3.2.18 Performance Characteristics

Not applicable

### 3.2.19 Supporting Documents

Sample collection manual, Quality manual, Safety manual

### 3.2.20 References

AAB technical manual 12$^{th}$ edition, AfSBTC 4$^{th}$ Edition.

## 3.3 PROCEDURE FOR COMPATIBILITY TESTING

### 3.3.1 Purpose

This procedure provides instructions for performing compatibility testing, which is used to select blood and blood components that will not cause harm to the recipient (patient)

### 3.3.2 Scope

This procedure is used in Blood Transfusion section when performing compatibility testing prior to issue of a unit of blood to recipient.

### 3.3.3 Responsible

The head of Blood Transfusion and competent medical laboratory personnel are responsible for ensuring this procedure is effectively implemented and maintained.

### 3.3.4 Principle

This method is based on immunophenotyping principle. The known red cell antigens from donor are mixed with unknown antibodies from the recipient (patient) to detect if there is any incompatibility caused by ABO, Rhesus and/or other blood groups antibodies.

### 3.3.5 Sample Requirements

- Patient/ recipient serum from a clotted blood sample in plain tube
- Donor cells from a segment tubing of the blood unit

### 3.3.6 Equipment

Centrifuge, Water bath, timer, refrigerator

### 3.3.7 Materials

| **Reagent** | **Consumables** |
|---|---|
| Incomplete anti-D (IgG)<br><br>Anti-Human Globulin<br><br>Serum (AGS)<br><br>Sensitized cell | Test tubes, Test tube rack, grease pencil,<br><br>Beakers,<br><br>Physiological Saline,<br><br>Pasteur pipettes |

### 3.3.8 Storage and Stability

Venous blood must be used within 3 hours at room temperature then after should be refrigerated at 2-8˚C. Blood Donor units should be stored at 1-6˚C

### 3.3.9 Safety

- Adhere to safety precautions as stated in the Safety manual
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections. iv. Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### 3.3.10 Calibration

Calibration should be done as per schedule.

### 3.3.11 Quality control

Quality Controls for Antihuman globulin should be done on a daily basis before performing patient samples..Internal quality control should be prepared from patient samples or known organisation.

### 3.3.12 Procedure Steps

#### *3.3.12.1 Immediate Spin Saline Technique*

i. Select a unit of blood from the blood bank storage with the same group as the recipient. If there is no unit with the same blood group as the recipient, blood group O packed red cells can be used as universal donor. Also recipients with AB blood group are considered as universal recipients of packed red cells.
ii. Label a tube for each donor red cell suspension being tested with the patient's serum.
iii. Add two drops of patient's serum or plasma to each tube.
iv. Add one drop of donorcells suspension into appropriate test tubes
v. Rinse the Pasteur pipette 5 times during transferring of cells and serum to avoid contamination
vi. Mix the contents of the tube(s) and centrifuge at 1000rpm for 60 seconds.
vii. Gently re-suspend the cell buttons and observe for haemolysis or agglutination.
viii. Read, interpret, and record test results. ix. If compatible, proceed with Indirect Antiglobulin Technique

#### *3.3.12.2 Interpretation and reporting:*

- Agglutination or haemolysis means a positive (incompatible) test results
- A smooth suspension of red cells after resuspension of the red cells button means negative results and indicates a compatible immediate spin cross match.

***Note: In emergency cases where the blood unit is required immediately, perform Immediate Spin Saline Technique. Issue the blood unit if it is compatible then proceed with Indirect Antiglobulin test. If there is any incompatibility, immediately call the ward to stop the transfusion and re-call the blood unit.***

### 3.3.13 Biological References Intervals

Not applicable

### 3.3.14 Reporting and Interpretation of results

#### *3.3.14.1 Interpretation of results*

Results should be reported in such a way that will indicate the recipient's blood group and the donor's blood unit number to which the donor's bloods is compatible or not compatible.

#### *3.3.14.2 Reporting of results*

Report as compatible when there is no agglutination or incompatible when there is no agglutination.

### 3.3.15 Limitation of the Procedure and Sources of Errors

- Haemolysis samples may lead to false negative results.
- Patients who have had recent multiple transfusions may develop allo-antibodies that can interfere with antigen – antibody reactions

### 3.3.16 Performance Characteristics

Refer to the method verification report.

### 3.3.17 Supporting Documents

Quality manual, sample collection manual and safety manual

### 3.3.18 References

Pam S. Helekar, D.P. Blackall et.al. American Association of Blood Bank 15 Edition, 1985. (method 3.1) and (method 3.2.1)

# CHAPTER FOUR: HAEMATOLOGY

## 4.1 PROCEDURE FOR SICKLING SCREENING TEST

### *4.1.1 Purpose*

The purpose of this procedure is to provide instruction for performing a screening test to determine abnormal type of Haemoglobin called Haemoglobin S in blood.

### *4.1.2 Scope*

This procedure is used by all trained laboratory staff while performing sickling test.in the Laboratory

### *4.1.3 Responsible*

Qualified, trained and competent Medical Laboratory Scientist, Technologist and Technician are responsible for doing this test procedure.

Section heads are responsible for ensuring the effective implementation and competency assessment for this procedure

### *4.1.4 Principle*

When a drop of blood is sealed between a cover slip and a slide, the decline in oxygen tension due to oxidative processes in the blood cells leads to sickling. In this method added with blood drop chemical reducing agents such as sodium met bisulphite. This rapidly reduces oxyhemoglobin to reduced haemoglobin, and then this will be accelerating sickling.

### *4.1.5 Sample Requirements*

3 - 4 ml of venous whole blood collected in EDTA tube (purple top vacuum). Sample must be free from haemolysis, lipemia and icterus.

### *4.1.6 Equipment*

Light Microscope, and Hot plate

### *4.1.7 Materials*

Freshly prepared 2% Sodium Metabisulphite (Diluting 0.2gm in 10ml of distilled water).

Vaseline/paraffin wax, Pipette, Cover glass, Glass slide and Applicator stick

### *4.1.8 Storage and Stability*

Processed whole blood is stable at 2$^{o}$C to 8$^{o}$C for 3 days.

Reagents are freshly prepared and stored at room temperature on daily basis. Do not use reagents that is more than 24 - hour post preparation.

### *4.1.9 Safety*

Adhere to safety precautions as stated in the Safety manual/IPC guideline All personal protective equipment (PPE) must be worn when performing this procedure.

All samples must be regarded as potentially infections.

### *4.1.10 Calibration*

Not Applicable

### *4.1.11 Quality Control*

Known positive control samples and negative control samples should be tested the same way as patient sample.

### *4.1.12 Procedural Steps*

i. Place one drop of the blood to be tested in a glass slide. ii. Add 1- 2 drops of freshly prepared 2% sodium met bisulphite to the drop of blood and mix well with an applicator stick.

iii. Place a cover glass on top of the sample and press down lightly on it to remove any air bubbles and to form a thin layer of the mixture. Wipe of the excess sample.

iv. Carefully rim the cover glass with molten paraffin wax or Vaseline, completely sealing the mixture under the cover slip.

v. Incubate for 24 hours at room temperature, or for one hour at $37^0$C. vi. Examine the prepared glass slide for the present of sickle cells after one hour using 40 X objective.

vii. If there is negative findings within one hour, allow the prepared slide to stand at room temperature for 24 hours, and examined under microscopy

***4.1.13 Biological Reference Interval*** Not applicable.

### *4.1.14 Interpretation and Reporting Of Results*

- Report **Positive** when the presence RBCs appear as moon shaped or shaped like a “C” showing they are sickle cells.
- Report **Negative** when the presence RBCs appear round Normal looking red cells
- The results should be interpreted along with other clinical features. Further tests might be necessary to confirm the disease condition.

### *4.1.15 Limitation of the Procedure and Sources of Errors*

Haemolysed samples. Iron deficiency or blood transfusions within the past 3 months can cause a false negative result

### *4.1.16 Performance Characteristics*

Refers to method verification report

### *4.1.17 Supporting Documents*

Sample collection manual

### *4.1.18 References*

Monica Cheesbrough Handwrite

## 4.2 PROCEDURE FOR URIT-12 HEMOGLOBIN METER

### *4.2.1 Purpose*

This procedure is used to describe step by step on how to operate the URIT-12 Haemoglobin Meter using human whole blood sample in the laboratory.

### *4.2.2 Scope*

This procedure is applied in testing hemoglobin parameter using human whole blood sample in the haematology department/section in the laboratory

### *4.2.3 Responsible*

A trained and competent laboratory scientist, laboratory technologists and assistant laboratory technologists are responsible for performing this procedure.

The head of section or assigned personnel will be responsible for ensuring that this procedure is effectively implemented.

### *4.2.4 Principle*

The URIT-12 Hemoglobin Meter utilizes optical reflectance for determination of the total hemoglobin. A drop of whole blood is applied to the test spot on the strip, blood immediately disperses within the membrane contacting the reagent, then reaction product could absorb spectrum in the range of 500nm – 600nm. The meter's optical detector automatically measures the change in membrane reflectance. The intensity of reflectance is inversely proportional to the hemoglobin concentration. The meter calculates and displays the total hemoglobin concentration in gram/decilitre (g/dL) in 12 seconds based on mathematical conversion.

### *4.2.5 Sample Requirements*

Fresh capillary or EDTA-anticoagulated venous whole blood

### *4.2.6 Equipment*

URIT-12 Hemoglobin Meter

### *4.2.7 Materials*

The materials required in this procedure are Clean gloves, Laboratory coats, Micropipettes, Cuvetes,Sharp container, Pricker and Alcohol swab

### *4.2.8 Storage and Stability*

Anticoagulated blood is stable up to 72 hours at 2-8 ◦c

### *4.2.9 Safety*

- Personnel Protective Equipment must be worn at all times
- All samples must be treated as potentially infectious.
- Adhere to safety precautions as stated in the Safety manual/IPC guidline

### *4.2.10 Calibration*

Not Applicable

### *4.2.11 Quality Control*

Use Hemoglobin HQ-A Control Solution or known higher and low concentration made in-houseto run on weekly basis to determine the accuracy of the patient results.

### *4.2.12 Procedural Steps*

**i.** Massage the patient's middle or ring finger from knuckle up to the tip to stimulate blood flow

**ii.** Insert the test strip into the strip holder with the notched end in first and the hole facing up. The notched end on the top of strip should no longer be visible when test strip is inserted correctly and fully

**iii.** Perform finger prick. Avoid "Milking" Apply light pressure to obtain one drop of blood.

**iv.** Take 13-15µl of whole blood with capillary tube or transfer pipette

**v.** Rapidly drip the blood into the sample spot on the strip when the meter shows blood symbol and ensure the test strip is covered by blood sample completely. vi. During the test do not disturb or move the meter or strip, even press any key of meter

vii. The test results will be displayed in less than 30 seconds

viii. Record the test results displayed on the machine. ix. Remove the test strip and immediately dispose off into highly infectious waste container.

### *4.2.13 Biological Reference Intervals*

Infant 14.0 - 22.0g/dl, Children 11.1 - 14.1g/dl, Adult male 13.0 -17.0g/dl, Adult female 12.0-15.0g/dl

### *4.2.14 Interpretation and Reporting Of The Results*

**Interpretation** of results
Interpretation of the results is based on the biological reference intervals.

**Reporting of results**
The obtained results will be reported in g/dl.

**Critical results:** HB ≤ (5.07) g/dl is considered as critical and communicate with clinician

### *4.2.15 Limitation of the Procedure and Sources of Errors*

Only whole blood or EDTA anticoagulated blood should be used.

### *4.2.16 Performance Characteristics*

Refer into method verification report

### *4.2.17 References*

URIT – 12 Hemoglobin meter Operation Manual

## 4.3 PROCEDURE FOR DETERMINATION OF HAEMOGLOBIN LEVEL USING HEMOCHROMAX PLUS

### *4.3.1 Purpose*

The procedure provides instructions to laboratory staff on operation of Hemochroma Plus Machine.

### *4.3.2 Scope*

This procedure is applicable when performing quantification of Haemoglobin (Hb) concentration in Hospital Laboratory.

### *4.3.3 Responsible*

Qualified, trained and competent Medical Laboratory Scientist, Technologist and Technician are responsible for doing this test procedure. Section heads are responsible for ensuring the effective implementation of this procedure.

### *4.3.4 Principle*

The hemochroma PLUS analyser utilizes a dual wavelength LED light sources by which the haemoglobin absorbance is detected and converted into an electrical signal. The signal is direct proportional to the amount of haemoglobin present in the blood sample.

### *4.3.5 Sample Requirements*

Whole blood, capillary or venous ant coagulated collected blood into EDTA tube.

### *4.3.6 Equipment*

Hemochroma PLUS machine

### *4.3.7 Materials*

Hemochromax Plus Micro calibrator cuvette, Calibrator ID chip cuvettes, , Gauze, 70% methylated spirit and Blood lancet or prickers

### *4.3.8 Storage and Stability*

Anticoagulated blood is stable up to 3 days at 2-8 ◦c

### *4.3.9 Safety.*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personnel must be worn protective equipment (PPE) when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.3.10 Calibration*

It should be done when the machine is not working properly or when provides a doubt result.

### *4.3.11 Quality Control*

Use commercial IQC materials or known higher and low concentration made inhouse to run on daily basis before performing the patient sample

### *4.3.12 Procedure*

i. Establish good relationship with the patient
ii. Make sure the patient is sitting comfortably
iii. Lightly massage to stimulate circulation. Only use the middle or ring finger.
The patient should not wear the ring on that finger iv. Pres lightly and draw finger-prick blood into a micro cuvette by bringing the cuvette in contact with the blood drop on the fingertip and puncture the side to a depth of cuvette.
v. Remove any excessive blood from the outside of the cuvette. vi. Insert the cuvette containing blood sample to the Hb machine

vii. Wait until displaying of the test results and record the findings into the system and register book
viii. Pull the cuvette holder out to its loading position and discard the used microcuvette in sharp box

### *4.3.13 Biological Reference Interval*

Infant 14.0 - 22.0g/dl, Children 11.1 - 14.1g/dl, Adult male 13.0 -17.0g/dl, Adult female 12.0-15.0g/dl

### *4.3.14 Interpretation and Reporting of Results*

**Interpretation** of results
Interpretation of the results is based on the biological reference intervals.

**Reporting of results**
The obtained results will be reported in g/dl.

**Critical results:** HB ≤ (5.07) g/dl is considered as critical and communicate with clinician

### *4.3.15 Limitation of the Procedure and Sources of Errors*

i. Only whole blood should be used

ii. Air bubbles in the optical eye caused by inadequate filling of the cuvettes may lead into false results

### *4.3.16 Performance Characteristics*

Refer to method verification report

### *4.3.17 Supporting Documents*

Sample Collection Manual, Safety Manual,

### *4.3.18 References*


- Monica Cheesbrough. District laboratory practice in tropical countries, Part 2. 2000.
- Hemochromax PLUS package insert
- Hemochromax PLUS user manual


## 4.4 PROCEDURE FOR DETREMANATION OF HEMOGLOBIN LEVEL USING HEMOCUE 201+ MACHINE

### *4.4.1 Purpose*

This procedure provides instructions for the performance of Haemoglobin Estimation using Hemocue 201+ machine.

### *4.4.2 Scope*

This procedure applies to all competent laboratory staffs during determination of haemoglobin level by using Hemocue 201+ machine.

### *4.4.3 Responsible*

Qualified, trained and competent health laboratory practitioners in the laboratory are responsible for implementation of this procedure.

### *4.4.4 Principle*

The reaction in the microcuvette is a modified azidemethemoglobin reaction. The erythrocytes are haemolysis to release the haemoglobin. The haemoglobin is converted to methoglobin and the combined with azide to form azide methoglobin. The measurement takes place in the analyser in which the transmittance is measured the absorbance and haemoglobin level is calculated. The absorbance is directly proportional the haemoglobin concentration

### *4.4.5 Sample Requirements*

Capillary whole blood sample or Anti-Coagulated Whole blood (collected in EDTA anticoagulant)

### *4.4.6 Equipment*

Hemocue 201+ machine

### *4.4.7 Materials*

Hemocue Hb 201+ microcuvette, Lancet/Pricker for capillary sample, Pipette or any other transfer device for venous sample or control materials

### *4.4.8 Storage and Stability*

Ant-Coagulated whole blood is stable up to 4 hours at room temperature and up to 24 hours at 4°C– 8°C.

### *4.4.9 Safety*

- Personnel Protective Equipment must be worn at all times
- All samples must be treated as potentially infectious.

### *4.4.10 Calibration*

Not Applicable

### *4.4.11 Quality Control*

The Hemocue Hb 201+ has an electronic self-test daily IQC or the use of known higher and low concentration made in-house can also be applied.

### *4.4.12 Procedural Steps*

i. Establish good relationship with the patient
ii. Make sure the patient is sitting comfortably
iii. Lightly massage to stimulate circulation. Only use the middle or ring finger. The patient should not wear the ring on that finger iv. Pres lightly on the fingertip and puncture the side to a depth of cuvette.
v. Remove any excessive blood from the outside of the cuvette. vi. Insert the cuvette containing blood sample to the Hb machine
vii. Wait until displaying of the test results and record the findings into the system and register book
viii. Pull the cuvette holder out to its loading position and discard the used microcuvette in sharp box

***Note: The microcuvette should be filled within 3 minutes after it has been taken out of its package.***

### *4.4.13 Biological reference Intervals*

Infant 17.0-22.0g/dl, Children 11.0-13.0g/dl, Adult male 13.0 -17.0g/dl, Adult female 12.0-15.0g/dl

#### *4.4.14 Interpretation and Reporting of Results*

**Interpretation** of results
Interpretation of the results is based on the biological reference intervals.

**Reporting of results**
The obtained results will be reported in g/dl.

**Critical results:** HB ≤ (5.07) g/dl is considered as critical and communicate with clinician

#### *4.4.15 Limitation of the Procedure and Sources of Error*

- Only whole blood should be used
- Air bubbles in the optical eye caused by inadequate filling of the cuvettes
- Delayement in transfer of filled cuvettes with blood into HB machine

#### *4.4.16 Performance Characteristics*

Refers into method verification report

#### *4.4.17 Supporting Documents*

Waste Management Procedure /IPC guideline Quality Control Result Procedure

#### *4.4.18 References:*

Monica Cheesbrough. District laboratory practice in tropical countries, Part 2. 2000.

Hemocue Hb 201+ cuvette kits insert and use manual.

### 4.5 PROCEDURE FOR CD 4 COUNT TEST BY USING BD FACS PRESTO

#### *4.5.1 Purpose*

The purpose of this procedure is to provide detailed information on how to analyse and detect CD4 T Cell enumeration on blood sample by using BD FACS presto.

#### *4.5.2 Scope*

This procedure applicable in haematology to analyse and detect CD4 T Cell by using BD FACS presto.

#### *4.5.3 Responsible*

Qualified, trained and competent Medical Laboratory Technician and Technologist are responsible for doing this test procedure. Section heads are responsible for ensuring the effective implementation and competency assessment for this procedure.

#### *4.5.4 Principle*

The BD FACS Presto™ cartridge the CD4/%CD4/Hb cartridge contains dried fluorochrome-conjugated antibody reagents. When blood reacts with the reagents, the antibodies in the reagent bind to the surface antigens on the BD FACS Presto Cartridge: lymphocytes and monocytes. After the incubation period, the cells are analysed on the BD FACS Presto Near-Patient CD4 Counter (the instrument). The software identifies the cell populations of interest and calculates CD4 absolute counts, CD4 percentages of lymphocytes, and haemoglobin concentration. The system measures total haemoglobin by spectrophotometric method, using absorbance at an isobestic point for oxyhemoglobin and deoxy-hemoglobin, with Correction for scatter.

### *4.5.5 Sample Requirements*

Blood sample on K2EDTA vacuum tubes. All K2EDTA samples must be received and set up within 24 hours from collection time.

### *4.5.6 Equipment*

BD FACS Presto

### *4.5.7 Materials*

| **Reagent** | **Consumables** |
|---|---|
| BD FACS presto cartridge | Blue and yellow tips<br>BD FACS presto print out paper<br>BD disposable 100 µl Pipette |

### *4.5.8 Storage and Stability*

- Do not refrigerate whole blood SAMPLE before sample preparation.
- Do not use previously fixed and stored samples.

### *4.5.9 Safety*

- Blood samples may contain infectious agents that are hazardous to your health. Observe Standard Universal precautions.
- Ensure the instrument and environment you working are kept clean and free from infectious substance such as human blood to avoid contamination.
- Spills should be immediately disinfected with 0.5% Sodium Hypochlorite Solution.

***4.5.10 Calibration*** Not Applicable.

### *4.5.11 Quality Control*

BD Facs presto has internal electronic self-test for IQC or the use of known higher and low CD4 counts made in-house can also be applied

### *4.5.12 Procedural Steps*

i. Open the cartridge package and label the patient ID on to the cartridge.

ii. Face the inlet port up. iii. Invert the tube 10 times to mix the contents well. iv. Use the pipette to obtain the sample.

v. Gently squeeze the bulb on the pipette to form a drop of blood on the tip of the pipette. vi. Carefully dispense the sample into the inlet port. Hold the cartridge by its ridges only.

vii. Make sure the blood reaches the top of the inlet port. If necessary, gently squeeze the bulb on the pipette to dispense more blood.

viii. Make sure the cartridge is level, with the barcode side up, at all times. Make sure that blood appears in the part of the channel not covered by the channel protector, next to the containment zone.

ix. Discard the pipette into a biohazard us waste container.

x. Close the cartridge cap securely and Set the on-board timer. xi. Place the cartridge, barcode side up, on the work station

xii. Press the Run Test tab. xiii. Press Patient ID.

xiv. Enter the patient's ID and press Accept. xv. The Confirmed Patient ID screen opens. xvi. Press Accept and Insert the cartridge:

xvii. Select your Operator ID and press Accept. Then cartridge door on the instrument opens will open.

xviii. Note: If possible, complete the following two steps within 30 seconds. xix. Remove the channel protector from the cartridge. xx. Hold the cap with the channel facing upwards.

xxi. Insert the prepared cartridge into the cartridge door. The cartridge door closes.

xxii. Press Accept to eject the cartridge within 30 seconds.

### *4.5.13 Biological Reference Interval*

| Analyte | Gender | Reference interval | SI UNIT |
|---|---|---|---|
| Absolute CD 4 count | Male | 462 – 1306 | cells/uL |
| | Female | 440 – 1602 | cells/uL |
| %CD4 of lymphocytes | Male | 29 – 54 | % |
| | Female | 32 – 55 | % |
| HAEMAGLOBIN | Male | 13.5 – 18.0 | g/dL |
| | Female | 12.0 – 16.0 | g/dL |

### *4.5.14 Interpretation and Reporting of Results*

**Interpretation of results**

- The results are displayed on the screen and print automatically.

**Reporting of Results**

- **R**eport the obtained results as displayed on the screen.

**Critical values**

- If the CD4 count is low (below 200cells/□l for adult and below 450 cells/ □l for children) the result will be regarded as critical and communicate with clinician

### *4.5.15 Limitations of the procedure and sources of error*

- Use the cartridge only with the BD FACS Presto instrument.
- The use of expired cartilage may result into false results
- Improper filling of the test device may not give the proper results

#### *4.5.16 Performance Characteristics*

Refer to Method verification report

#### *4.5.17 Supporting Documents*

Quality Manual, Sample Collection Manual, Safety Manual.

#### *4.5.18 References*

Becton Dickinson BD FACS presto Operating Manual

### 4.6 PROCEDURE FOR DETERMINATION OF CLOTTING TIME

#### *4.6.1 Purpose*

The purpose of this standard operating procedure (SOP) is to provide guidelines to be followed for performing clotting time

#### *4.6.2 Scope*

This procedure is to be performed at point of care or any health facility to detect the clotting time

#### *4.6.3 Responsible*

The Section heads are responsible section is responsible for ensuring the effective implementation and maintenance of this procedure. Qualified, competent and registered Medical Laboratory practitioners are responsible for implementing this test procedure.

#### *4.6.4 Principle*

The presence of activator augments the contact activation phase of coagulation, which stimulates the intrinsic coagulation pathway. Clotting time can be performed manually, whereby the operator measures the time interval from when blood is injected into the test tube to when clot is seen along the sides of the tube.

#### *4.6.5 Sample Requirements*

Whole blood

#### *4.6.6 Equipment*

Stop watch, water bath and thermometer

#### *4.6.7 Materials*

Disposable gloves, Laboratory coat, 70% alcohol, Masks, and Sterile lancets

### *4.6.8 Storage and Stability*

Not applicable

### *4.6.9 Safety*

- Adhere to safety precautions as stated in the facility Safety manual
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.
- Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### *4.6.10 Calibration*

- Perform equipment calibration of the Stop watch, water bath and thermometer as per auxiliary equipment calibration schedule

### *4.6.11 Quality Control*

Not Applicable

### *4.6.12 Procedural Steps*

Two methods can estimate clotting time:

❑ **Capillary method of bleeding time.**

i. Prick the finger with the lancet. ii. Hold the capillary over the blood, and the capillary will fill automatically.

iii. Now, after regular intervals, break the capillary. iv. When a clot starts forming, that is the endpoint and clotting time. ❑ **Test tube method of clotting time.**

i. Perform this test at 37 ° C.
ii. Take 4 ml of blood for the tube method and start the time.
iii. Note the time when there is the first appearance of the clot formation.
iv. This test can be done in multiple tubes to be more accurate.

### *4.6.13 Biological Reference Interval*

Not Applicable

### *4.6.14 Interpretation and Reporting of Results*

**i.** The expected range is 4 to 10 minutes.

**ii.** The glass tube method clotting time is 5 to 15 minutes. **iii.** Results are given in amount of minutes takes for bleeding time to stop

### *4.6.15 Limitation of the Procedure and Sources of Error*

**i.** This test is only prolonged in severe deficiency.

**ii.** Normal clotting time is despite prolonged bleeding time seen in thrombocytopenia.

**iii.** This may be normal in patients taking anticoagulant therapy.

**iv.** This is usually normal when the intrinsic and common pathways are present in an amount not exceeding 1% of the normal plasma level.

### *4.6.16 Performance Characteristics*

Refer the method verification report of the procedure.

### *4.6.17 Supporting Documents*

Sample collection manual

### *4.6.18 References*

A manual of laboratory diagnostic tests. Edition 7, Lipipicontt William and Wilkins, by Frances Talaska Fishbach, RN, BSN, MS, and MarshallaBrnet 11, RN, BSN, MS, Ph.D.

## 4.7 PROCEDURE FOR DETERMINATION OF THE BLEEDING TIME

### *4.7.1 Purpose*

The purpose of this procedure is to provide instructions for investigation of bleeding time.

### *4.7.2 Scope*

This procedure is used in Haematology section when performing bleeding time

### *4.7.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head Haematology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.7.4 Principle*

Bleeding time is a medical test done to assess platelet function of a patient. It involves cutting the underside of the subject's forearm, in an area where there is no hair or visible veins. The cut is of a standardized width and depth, and is done quickly by an automatic device. A blood pressure cuff is used above the wound, to maintain venous pressure at a special value. The time it takes for bleeding to stop (i.e. the time it takes for a platelet plug to form) is measured. Cessation of bleeding can be determined by blotting away the blood every several seconds until the site looks 'glassy'.

### *4.7.5 Sample Requirements*

Plasma/whole blood, Serum (2-5ml)

### *4.7.6 Equipment*

Timer, Thermometer, Light Microscope, Sphygmomanometer

### *4.7.7 Materials*

Marker pen, Examination Gloves, Filter paper, 70% alcohol swabs and Sterile lancets

### *4.7.8 Storage and Stability*

Not Applicable

### *4.7.9 Safety*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.7.10 Calibration*

Perform calibration of equipment (Timer and Thermometer) as per calibration schedule

### *4.7.11 Quality Control*

Not Applicable

### *4.7.12 Procedural Steps*

i. Apply the blood pressure cuff to the arm just above the elbow. ii. Inflate the device to 40 mm of mercury and maintain at this level. iii. Clean the anterior surface of the fore arm with 70% alcohol swabs.

iv. Make two clean punctures about 2 mm long and 2 mm deep being careful to avoid underlying veins.

v. Blot the blood with the filter paper every 15 seconds but be careful and make sure you only touch the top of the drop.

vi. start the stop watch as the first drop of the blood appears, immediately when the blood ceases stop the timer and record the time.

vii. Calculate the average of the 2 punctures and record bleeding time.

viii. **If** it's a prolonged bleeding time **then** perform Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and platelets count.

### *4.7.13 Biological Reference Interval*

Not Applicable

### *4.7.14 Interpretation and Reporting of Results*

**Interpretation** of results

i. Interpret results in terms of minutes taken for bleeding to stop. ii. Normal ranges are around 1½ - 5 minutes.

***NOTE; If the bleeding time is greater than 10 minutes, stop the test and apply pressure to the wound. Report the results as greater than 10 minutes.***

**Reporting of results**

Report results as: *Bleeding time (in minutes)*

**Critical value**

Findings *greater than 10 minutes.*

### *4.7.15 Limitation of the Procedure and Sources of Error*

Anything that alters platelet function can interfere with the bleeding time. Some examples include aspirin, thrombocytopenia, and uremia

### *4.7.16 Performance Characteristics*

Not Applicable

### *4.7.17 Supporting Document*

Sample collection manual

### *4.7.18 References*

## 4.8 PROCEDUIRE FOR FULL BLOOD COUNT BY USING URIT BH – 40P HAEMATOLOGY ANALYSER.

### *4.8.1 Purpose*

This procedure provides instruction on how to operate urit BH-40P haematology analyser in determining full blood count

### *4.8.2 Scope*

This procedure applies in the Haematology section when performing FBC (Full blood count) analysis using URIT BH - 40P Haematology Analyser.

### *4.8.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this procedure. The Head Haematology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.8.4 Principle*

The URIT BH – 40P Automated Haematology Analyser is a multi-parameter, it can display 21 parameters and 3 histograms. Analyser adopts electrical impedance method for WBC, RBC and PLT test and colorimetric method for HGB test. The electrical impedance method is based on non-conductivity of blood cells. When the blood cells in diluents pass through the ruby aperture, resistance will change, based

on that we can get the counting and volume of blood cells. The colorimetric methods to measure and calculate HGB. Add lyse into the diluents sample, and then RBC will be dissolve and release haemoglobin. Then the haemoglobin combines with lyse to form cyanohemoglobin. Measure the transmission light intensity of this compound in a sample cup through the monochromatic light of 540 nm wavelength and then compare it with the result in blank state to get the haemoglobin concentration (blank state refers to the state that only has diluents in sample cup).

### *4.8.5 Sample Requirements*

Whole blood sample in EDTA-K2.2H2O tube.

### *4.8.6 Equipment*

- URIT BH-40 Automated Haematology Analyser
- Perform start up, maintenance, troubleshooting and shut down the URIT BH-40 Automated Haematology analyser as per manufacturer's instrument instructions

### *4.8.7 Materials*

Diluent, Lyse, Probe detergent, Set of Controls kit, Marker pen, Examination Gloves Capillary tube, Plane test tube, Thermal paper, and Gloves

### *4.8.8 Storage and Stability*

- Keep the Set of Controls at $2^{o}C$ – $8^{o}C$
- Never use reagent and control beyond its expiration date
- Blood sample be kept at temperature between $2^{o}C$- $8^{o}C$ for 7 days.

### *4.8.9 Safety*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.8.10 Calibration*

- URIT calibrates the analyser in factory before shipment.
- Use the user URIT BH-40P to recalibrate the analyser when there is shifts or trends in some parameters

### *4.8.11 Quality Control*

Run all quality controls; QC1 (Low), QC 2 (Normal) and QC 3 (High) before examination of patient samples

### *4.8.12 Procedural Steps* **Running Patient Samples**

**i. Pre- diluent peripheral blood mode**

- Present the empty sample tube under aspiration probe.

- At main menu screen, click “Drain”; the diluents will be dispensed into the tube.
- Remove the tube, add 20µl of the blood sample to the tube, and gently shake the tube to make them well mixed.
- After that present the well mixed sample under the aspiration probe; make sure the probe touches bottom slightly.
- Press RUN key on the front panel and remove the sample after hearing beep sound. The result will be available after analysis is performed. **ii. Whole Blood Mode**
- Gently shake the tube to well mix the blood sample, then present the sample tube beneath the probe, make sure the probe touches tube bottom slightly.
- Press RUN key and remove the sample after hearing beep sound. The results will be available after analysis is performed.

**iii. Ant coagulated Peripheral Blood Mode**

- Gently shake the tube to well mix the blood sample, then present the sample tube beneath the probe, make sure the probe touches tube bottom slightly.
- Press RUN key and remove the sample after hearing beep sound. The results will be available after analysis is performed.

### *4.8.13 Biological Reference Interval*

See annex 1.

### *4.8.14 Interpretation and Reporting of Results*

**Interpretation** of results

- Normal: if the results are within acceptable range.
- Abnormal (pathological): if the results are out of acceptable range

**Reporting of results**

Results are automatically printed from the URIT BH-40P and then review by section head. Critical value

WBC > 20 $x10^9$/L, HGB <5g/dL, PLT < 50 or 1000 $x10^9$/L

### *4.8.15 Limitation of the Procedure and Sources of Error*

- The test will be affected by hemolysed blood and coagulated blood.
- Samples with extreme lipemic, chylomicrons or extremely high bilirubin concentrations might produce falsely elevated haemoglobin values.

### *4.8.16 Performance Characteristics*

Refer to the method verification report of this procedure.

### *4.8.17 Supporting Document*

Sample collection manual

### *4.8.18 References*

## 4.9 PROCEDURE FOR FULL BLOOD COUNT USING OF ABX PENTRA 80 HAEMATOLOGY ANALYSER

### *4.9.1 Purpose*

This procedure provides instructions for operation and maintenance of ABX Pentra 80 analyzer for Full blood picture

### *4.9.2 Scope*

This procedure applies to all Full Blood Count tests done on ABX Pentra 80 analyser in the haematology section

### *4.9.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head Haematology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.9.4 Principle*

The ABX Pentra 80 is an automated haematology analyser used for counting and differentiating the cellular components in whole blood using electrical impedance, cytochemical staining, light scatter and spectrophotometer.

The principle behind cell counting is based on disruption of electric current as particles pass through an orifice. An electric current applies on both sides of this orifice. Cells do not conduct electric current, therefore their passage through the orifice leads to a change of the electric current established between both electrodes. These electric current differences are registered and increment a counter at every cell passage.

A chemical agent is used to separate erythrocyte and leukocyte populations, because of size overlapping and quantities discrepancies. This chemical agent contained in Lysis (ACTI-DIFF) pops the cytoplasmic membrane of the red cells. Erythrocyte population disappears leaving the leukocytes.

A haemoglobin preservative is added in lysing agent to measure haemoglobin scaled down in a 540nm photometric tank at the end of the counting. The haemoglobin measurement is made from the first dilution. The lysing agent has a powerful

haemoglobin reducer (potassium cyanide) and then the haemoglobin measurement follows Drabkin method with a 540nm reading. The integration of luminous intensity transmitted is evaluated according to the BEER-LAMBERT formula. An enzymatic liquid (ABX CLEANER) ensures the system cleanliness between every analysis and prevents carryover between samples.

### *4.9.5 Sample Requirements*

2 to 4mls of whole blood collected in K3 EDTA tube

### *4.9.6 Equipment*

ABX Pentra 80 Analyser and Perform start up, maintenance, troubleshooting and shut down the ABX Pentra 80 Haematology analyser as per manufacturer's instrument instruction.

### *4.9.7 Materials*

ABX Pentra 80 analyser reagent pack, Laboratory coat, Biohazard waste container. 0.5% Sodium hypochlorite solution, Distilled water, Protective gloves, Methanol, A4 paper

### *4.9.8 Storage and Stability*

Control kit is stable until expiry date and should be kept at $2^oC$ – $8^oC$. Blood sample be kept at temperature between $2^oC$- $8^oC$ for 7 days.

### *4.9.9 Safety*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.9.10 Calibration*

Calibrate the ABX Pentra 80 analyser under the following conditions:

- Change of software
- Major component replacement

### *4.9.11 Quality Control*

Run control QC materials (Low, Normal and High) before patient samples

### *4.9.12 Procedural Steps*

i. Click the STAT MODE key.
ii. Then write the patient ID, Age, Gender.
iii. Click the VALIDATE key.
iv. Mix well the patient sample.

v. Place the sample on the tube holder of analyser.
vi. Press the door of analyser inside to run the sample.
vii. The patient results will be printed automatically.

### *4.9.13 Biological Reference Interval* See annex 1.

### *4.9.14 Interpretation And Reporting Of Results*

- Normal: if the results are within acceptable range.
- Abnormal (pathological): if the results are out of acceptable range
- Results are automatically printed from the ABX Pentra 80 and then review by section head.
- Communicate the Critical value with clinicians

| Test | Critical Value |
|---|---|
| **Haemoglobin** | Less than 5 g/dl (50 g/L) |
| **White Blood Count** | Less than 2.0 or greater than 18.0 cells/$mm^3$ |
| **Platelet Count** | Less than 50,000/ $mm^3$ or greater than 800,000/$mm^3$ |

### *4.9.15 Limitation of the Procedure and Sources of Error*

i. Sickled red blood cells may not be accurately recognised and may give erroneous results
ii. Samples with cold agglutinins may falsely decrease the red cell count. The indices will indicate that the haemoglobin and haematocrit values do not agree. Thin film is recommended. These samples should be incubated for 30 minutes at 37 $^oC$ and reanalysed.
iii. Platelet clumps and neonatal samples may interfere with Drabkins method of haemoglobin determination.
iv. Samples with extreme lipaemia, chylomicrons or extremely high bilirubin concentrations might produce falsely elevated haemoglobin values.
v. Samples with nucleated red blood cells may falsely elevate white cell count. Additionally, presence of nucleated red cells may interfere with white cell differential count. Samples from patients with elevated chylomicrons ad those receiving total parenteral nutrition (TPN) including a high lipid concentration may falsely elevate the platelet count.
vi. Aggregated platelets may falsely elevate the white blood cell count and percentage of lymphocytes.
vii. The presence of immature white blood cells, including blasts, may affect the accuracy of the differential. The instrument will give an << I >> or 'M' error code if the blasts are suspected. A thin film is recommended.
viii. Clinical studies have shown that the Full Blood Count is not affected by presence of malaria parasites, Howell-Jolly bodies, cryogoblins and red cell fragments.

### *4.9.16 Performance Characteristics*

Refer to the method verification report of this procedure.

### *4.9.17 Supporting Document*

Sample collection manual

### *4.9.18 References*

ABX PENTRA 80 operator's manual

## 4.10 PROCEDURE FOR FULL BLLOD COUNT USING SINNOWA HB - 7021

### *4.10.1 Purpose*

This procedure provides instructions for operation and maintenance of ***SINNOWA HB – 7021 Hematology Analyser.***

### *4.10.2 Scope*

This procedure applies to all Full Blood Count tests done on SINNOWA HB – 7021 analyser in the haematology section

### *4.10.3 Responsible*

- Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.
- The Haematology section head is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.10.4 Principle*

The instrument adopts the method of impedance to measure and count cells. The conductivity liquid (mainly diluents) provides constant current source to the electrode thus they circuit can form a steady impedance circulation. When cells pass through the aperture, the conductive liquid is replaced by cells. Change of circuit resistance produces electrical pulse. The amplitude varies when the cells of different size pass through the aperture. Consequently the number and volume of cells pass through the aperture can be calculated based on the amplitude.

### *4.10.5 Sample Requirements*

2 to 4mls of whole blood collected in K3 EDTA tube

### *4.10.6 Equipment*

- SINNOWA HB – 7021 Haematology Analyzer, Refrigerator
- Perform start up, maintenance, troubleshooting and shut down the SINNOWA HB – 7021 Haematology analyser as per manufacturer's instrument instruction

### *4.10.7 Materials*

| **Reagents** | **consumables** |
|---|---|

| | |
|---|---|
| SINNOWA Diluent Reagent, SINNOWA Lyse Reagent, SINNOWA Detergent, SINNOWA Probe Detergent, | Laboratory coat, Biohazard waste container, 0.5% Sodium hypochlorite solution, Distilled water, Protective gloves, Methanol, Printing paper |

### *4.10.8 Storage and Stability*

- Control kit is stable until expiry date and should be kept at 2$^{o}$C – 8$^{o}$C
- Blood sample be kept at temperature between 2$^{o}$C- 8$^{o}$C for 7 days.
- Store reagents as per manufacturer instructions

### *4.10.9 Safety*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.10.10 Calibration*

The SINNOWA HB – 7021Hematology Analyser System requires commercial calibrator material or assayed whole blood for calibration.

Calibrate the SINNOWA HB – 7021Hematology Analyser under the following conditions:

- Change of software
- Major component replacement

***4.10.11 Quality Control*** • Run the three levels of control LOW, NORMAL and HIGH" for "SINNOWA HB – 7021 Hematology Analyzer to ensure quality of results

Perform Quality Control:-

i. Before analyzing the samples
ii. After replacement of the reagents
iii. After maintenance component replacement, or a field service action
iv. If there is any doubt in accuracy of the test results
v. After a reagent lot number change
vi. After a software change
vii. Following calibration
viii. According to your laboratory's quality control program
ix. According to manufacturer requirements

### *4.10.12 Procedural Steps*

i. Turn on the power on rear panel and indicator shows red light
ii. The instrument initializes test program
iii. Diluents, lyses and detergent will be sucked and tubing system cleaned
iv. If initialization is finished the display shows all parameters WBC, RBC and PLT

v. Shift to select ID and then press OK
vi. Select the test selection from the drop down menu
vii. Gently mix the sample
viii. Open the sample tube and place it under the Open Mode Probe
ix. Raise the tube until the end of the probe is deeply immersed in the sample.
x. Press the Touch Plate to activate aspiration
xi. Remove the tube when the beep sounds and replace the cap.
xii. When the cycle is finished, the results post to the Data log and are displayed in the Run View
xiii. Print the results

### *4.10.13 Biological Reference Interval* See annex 1.

### *4.10.14 Interpretation and Reporting of Results*

- Normal: if the results are within acceptable range.
- Abnormal (pathological): if the results are out of acceptable range
- Results are automatically printed from the SINNOWA HB – 7021 and then review by section head.
- Communicate the Critical value with clinicians

| Test | Critical Value |
|---|---|
| **Haemoglobin** | Less than 5 g/dl (50 g/L) |
| **White Blood Count** | Less than 2.0 or greater than 18.0 cells/$mm^3$ |
| **Platelet Count** | Less than 50,000/ $mm^3$ or greater than 800,000/$mm^3$ |

### *4.10.15 Limitation of the Procedure and Sources of Error*

- Keep reagent away from direct sunlight and protect them from evaporation.
- Use reagent container cap attached to each inlet tube, the cap will minimize evaporation and contamination.
- Never use reagent, control and calibrators beyond their expiration date.

### *4.10.16 Performance Characteristics*

Refer to the method verification report of this procedure.

### *4.10.17 Supporting Document*

Sample collection manual

### *4.10.18 References*


- SINNOWA HB – 7021 Hematology Analyser user manual.


## 4.11 PROCEDURE FOR FULL BLOOD COUNT USING BHA 3000 HAEMATOLOGY ANALYSER

### *4.11.1 Purpose*

This procedure provides instructions for operation and maintenance of BHA 3000 Haematology Analyser.

### *4.11.2 Scope*

This procedure applies in haematology section for performing Full Blood Count tests done on BHA 3000 analyser

### *4.11.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure. The Haematology section head is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.11.4 Principle*

BHA -3000 Automatic Hematology Analyser provides a 3 part differential blood count uses an electrical impedance to count red blood cells, platelets and volume distributions, and uses calorimetry to measure the haemoglobin and relevant parameters will be enumerated.

This system uses electrical impedance method to count red blood cells, platelets and white blood cells. When the absorbed quantitative sample is diluted by a quantitative conductive solution, it is sent to the detection unit of the instrument. The detection unit has a detection aperture, with a pair of positive and negative electrodes on both sides of the aperture, which is connected with a constant current power supply. Due to the bad conductor characteristic of these cells, when the cells in the diluted sample pass through the detection aperture under a constant negative pressure, the DC resistance between the electrodes will change, thus forming a pulse change at both ends of the electrode in portions to the size of the cell volume. When cells continuously pass through the aperture, a series of electrical pulses are generated at both ends of the electrodes. The number of pulse is equal to the number of cells passing through the aperture. And the pulse amplitude is proportion to the cell volume. Amplify the collected electrical pulses, and calculates the number of electric pulse in the red blood/platelets channel and WBC channel. The pulses are then classified according to different channel voltage threshold, hence determines the cell volume distribution

### *4.11.5 Sample Requirements*

2 to 4mls of whole blood collected in $K_3$ EDTA tube

### *4.11.6 Equipment*

BHA 3000 Haematology Analyzer, Refrigerator

### *4.11.7 Materials*

DIL-3 Diluent and HR-1 Lyse, Controls (Normal level (N), Low- level (L) and High- level (H) Laboratory coat, Biohazard waste container. 0.5% Sodium hypochlorite solution, Distilled water, Protective gloves, Printing paper

### *4.11.8 Storage and Stability*

- Control kit is stable until expiry date and should be kept at $2^oC$ – $8^oC$
- Blood sample be kept at temperature between $2^oC$- $8^oC$ for 7 days.
- Store reagents as per manufacturer instructions

### *4.11.9 Safety*

- Adhere to safety precautions as stated in the Safety manual/IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *4.11.10 Calibration*

Calibrate the BHA 3000 Haematology Analyzer Analyser under the following conditions:

- Change of software
- Major component replacement

### *4.11.11 Quality Control*

Run the three levels of control LOW, NORMAL and HIGH" for BHA 3000 Haematology Analyzer to ensure quality of results

Perform Quality Control:

i. Before analyzing the samples
ii. After replacement of the reagents
iii. After maintenance component replacement, or a field service action
iv. If there is any doubt in accuracy of the test results
v. After a reagent lot number change
vi. After a software change
vii. Following calibration
viii. According to your laboratory's quality control program
ix. According to manufacturer requirements

### *4.11.12 Procedural Steps*

i. Turn on the power on rear panel and indicator shows red light
ii. The instrument initializes test programme
iii. Diluents, lyses and detergent will be sucked and tubing system cleaned

iv. If initialization is finished the display shows all parameters WBC,RBC and PLT
v. Shift to select ID and then press OK
vi. Select the test selection from the drop down menu
vii. Gently mix the sample
viii. Open the sample tube and place it under the Open Mode Probe
ix. Raise the tube until the end of the probe is deeply immersed in the sample.
x. Press the press Plate to activate aspiration
xi. Remove the tube when the beep sounds and replace the cap.
xii. When the cycle is finished, the results post to the Data log and are displayed in the Run View
xiii. Print the results

### *4.11.13 Biological Reference Interval* See annex 1.

### *4.11.14 Interpretation and Reporting of Results*

- Normal: if the results are within acceptable range.
- Abnormal (pathological): if the results are out of acceptable range
- Communicate the following Critical value with clinicians

| Test | Critical Value |
|---|---|
| **Haemoglobin** | Less than 5 g/dl (50 g/L) |
| **White Blood Count** | Less than 2.0 or greater than 18.0 cells/$mm^3$ |
| **Platelet Count** | Less than 50,000/ $mm^3$ or greater than 800,000/$mm^3$ |

### *4.11.15 Limitation of the Procedure and Sources of Error*

- Keep reagent away from direct sunlight and protect them from evaporation.
- Use reagent container cap attached to each inlet tube, the cap will minimize evaporation and contamination.
- Never use reagent, control and calibrators beyond their expiration date.

### *4.11.16 Performance Characteristics*

Refer to the method verification report of this procedure.

### *4.11.17 Supporting Document*

Sample collection manual

### *4.11.18 References*

BHA 3000 Haematology Analyser user manual.

## 4.12 PROCEDURE FOR PERFORMING FULL BLOOD COUNT BY USING MS4 HAEMATOLOGY ANALYSER

### *4.12.1 Purpose*

This procedure provides instructions for operation and maintenance of MS4 analyser for Full blood count

### *4.12.2 Scope*

This procedure applies to all Full blood count tests done on MS4 analyser in the Laboratory hematology section

### *4.12.3 Responsible*

- Qualified and trained Health Laboratory Practitioners are responsible for implementing this test procedure.
- Section head is responsible for ensuring the effective implementation and maintenance of this procedure.

### *4.12.4 Principle*

The MS4-S is an automated haematology analyser used for counting and differentiating the cellular components in whole blood using electrical impedance, cytochemical staining, light scatter and spectrophotometer.

The principle behind cell counting is based on disruption of electric current as particles pass through an orifice. An electric current applies on both sides of this orifice. Cells do not conduct electric current, therefore their passage through the orifice leads to a change of the electric current established between both electrodes. This electric current difference is registered and increment a counter at every cell passage.

A chemical agent is used to separate erythrocyte and leukocyte populations, because of size overlapping and quantities discrepancies. This chemical agent contained in Lysis (ACTI-DIFF) pops the cytoplasmic membrane of the red cells. Erythrocyte population disappears leaving the leukocytes. A Haemoglobin preservative is added in lysing agent to measure Haemoglobin scaled down in a 540nm photometric tank at the end of the counting. The Haemoglobin measurement is made from the first dilution. The lysing agent has a powerful Haemoglobin reducer (potassium cyanide) and then the Haemoglobin measurement follows Drabkin method with a 540nm reading. The integration of luminous intensity transmitted is evaluated according to the BEER-LAMBERT formula.

An enzymatic liquid (TRANSFLUX) ensures the system cleanliness between every analysis and prevents carryover between samples.

### *4.12.5 Sample Requirements*

2 to 4mls of whole blood collected in K3 EDTA purple top color tube

### *4.12.6 Equipment*

MS4-S Hematology Analyser, and roller mixer

### *4.12.7 Materials*

MS4-S analyser reagent pack, Lab coat, Biohazard waste container, 5-6% Sodium hypochlorite, Distilled water, Protective gloves and Methanol

### *4.12.8 Storage and Stability*

Blood is stable for about 4 hours at room temperature or 24hours at 2- 6$^{o}$C

### *4.12.9 Safety*

i. Treat all samples as potentially infectious.
ii. Keep hands away from the sample carrier when the analysis begins
iii. Some components inside the MS4s have sharp edges or angular corners, therefore operate with caution to avoid cuts to the hands
iv. When performing maintenance procedure, take similar precautions as you would take when handling patient samples

### *4.12.10 Calibration*

All auxiliary equipment should be calibrated annually

### *4.12.11 Quality Control*

Use commercially quality control or in house prepared quality control samples

**Running of quality control**

i. Take the QC material from the refrigerator and let them stay at room temperature for 15 minutes.
ii. After 15 minutes' mix well the controls one by one by inverting the tube gently at least (x7) seven times without creating bubbles.
iii. Press ANALYSIS key (Tube like symbol) then DOWN arrow (↓).
iv. Select Quality CT (to run controls) then use RIGHT arrow (→ ) to select control levels.
v. Select LOW if you start with low control up to High control using UP and DOWN arrows on key board or on machine. (↑) or (↓)
vi. Place the control tube on the tube holder
vii. Press ENTER key on keyboard or (→) on the machine to validate
viii. Repeat the procedure to the Normal and High controls

ix. The QC results will be printed automatically
x. Plot the results of the QC on the Levy-Jennings chart and verify that the QC has passed before running patients' samples.

### *4.12.12 Procedural Steps*

**Running of Patient Samples**

i. Press Analysis key in the machine
ii. Press RIGHT arrow ⟶
iii. Select the gender female or male by using UP and DOWN arrows (↑) or (↓)
iv. Press the ENTER key (using key board) or (⟶) on the machine to validate
v. Write the name of the patient (Full Name)
vi. Mix well the sample
vii. Place the sample on the tube holder of the machine.
viii. Press ENTER on keyboard or (⟶) on the machine to start running the sample.
ix. The patient results will be printed automatically.

### *4.12.13 Biological Reference Intervals*

See annex 1.

***Critical value/results*** *should be recorded and immediately communicated to the requesting clinician.*

### *4.12.14 Interpretation and Reporting of Results*

Results are reported and communicated through the appropriate locally established procedure

### *4.12.15 Limitation of the Procedure and Sources of Errors*

i. Exposing reagents to direct sunlight may cause them to deteriorate
ii. Sickled red blood cells may not be accurately recognised
iii. Samples with cold agglutinins may falsely decrease the red cell count.
iv. Platelet clumps and neonatal samples.
v. Samples with extreme lipaemia, chylomicrons or extremely high bilirubin concentrations might produce falsely elevated Haemoglobin values.
vi. Samples with nucleated red blood cells may falsely elevate white cell count.
vii. Samples from patients with elevated chylomicrons and those receiving total parenteral nutrition (TPN) including a high lipid concentration may falsely elevate the platelet count.
viii. Circulating micro megakaryocytes may be counted as white cells
ix. Aggregated platelets may falsely elevate the white blood cell count and percentage of lymphocytes.

### *4.12.16 Performance Characteristics*

Method verification should be carried out and its report will be referred to fulfil this requirement.

### *4.12.17 Supporting Documents*

Laboratory quality policy manual, Laboratory safety policy manual and Laboratory sample collection manual

### *4.12.18 References*

MS4S operator's manual, 2013-07

# CHAPTER 5: CLINICAL CHEMISTRY AND IMMUNOLOGY

## 5.1 PROCEDURE FOR BLOOD GLUCOSE BY USING ACCU-CHECK GLUOCOMETER

### *5.1.1 Purpose*

This procedure provides instructions for use of blood glucose test strips *for* determination of Blood Glucose level. Blood glucose is measured mainly in the diagnosis and maNot applicablegement of diabetes mellitus.

### *5.1.2 Scope*

This procedure will be used for Blood Glucose testing in the laboratory and at point of care testing sites (POCT).

### *5.1.3 Responsible*

Qualified and competent registered and licensed Health laboratory practititioners and Trained health care providers respectively are responsible for doing this test procedure.

The head of clinical chemistry section is responsible for ensuring the effective implementation of this procedure.

### *5.1.4 Principle*

The test strips contain a capillary that sucks up a reproducible amount of blood. Glucose in the blood reacts with an enzyme electrode containing Glucose oxidase (or Glucose dehydrogeNot applicablese).The enzyme is reoxidized with an excess of a mediator ferricyanide ion, a ferrocene derivative or osmium bipyridyl complex. The mediator in turn is reoxidised by reaction at the electrode, which generates an electrical current. The total charge passing through the electrode is proportioNot applicablel to the amount of glucose in the blood that has reacted with enzyme.

### *5.1.5 Sample requirements*

i. Capillary or 1ml Fluoride-oxalate venous anticoagulated blood (fasting, post-prandial, or random samples). Do not collect blood from an arm receiving an I.V. infusion. *Fasting samples:* This refers to blood collected after a period of no food intake. For adults the fasting time is usually 10 to 16 hours. For children the fasting time is 6 hours unless a longer time is indicated, e.g. when investigating hypoglycaemia. The drinking of plain water is permitted.

ii. *Post-prandial samples:* This describes blood collected after a meal has been taken. The sample is usually taken as a 2 hour post - prandial samples.

iii. *Random samples:* This refers to a blood sample collected at any time, regardless of food intake.

***5.1.6 Equipment*** Glucometer **Maintenance**

Conduct **maintenance** as required by manufacturer instructions

***5.1.7 Materials***

Glucose test strips, Lancets, Cotton wool or gauze or alcohol swab, Sharp box or Container, waste bin, Disposable gloves, Laboratory coat

***5.1.8 Storage and stability***

All related materials should be stored as the per manufactures instructions. Sample should be processed within 1hour after collection

***5.1.9 Safety***

i. All samples must be considered as potentially infectious and must be handled and examined with care. ii. All person applicable protective equipment (PPE) should be worn when performing procedure

iii. Adhere to safety precautions as stated in the Safety manual

Refer to National infection prevention and control Guidelines for health waste management and safety practice.

***5.1.10 Calibration***

Equipment should be calibrated as per schedule.

***5.1.11 Quality control***

Process internal quality control before examing the patient samples on daily base

***5.1.12 Procedure Steps***

i. Compare the code number on the chip with the corresponding code number on the label of the test strip container where applicable. ii. The three-digit number on the code chip (e.g.689) must match the three-digit number on the label. (Leave the meter turned off).

iii. Gently slide the code chip into the slot on the side of the meter. (You must feel the code lock into place)

iv. To turn on the Glucose meter, press the S button and hold it down for more than 3 seconds until the depicted display appears.

v. Wear gloves clean the patient's finger using the alcohol swab and allows it to dry.

vi. Take one strip from the container. Close cap tightly and make sure the yellow color in the round window on the back of the test strip matches the yellow color above 0 mg/dL on the container. If it looks green do not use it.

vii. Insert the test strip, with the orange pad facing up, until it will go no further into the meter. Do not bend the test strip. (The arrow heads are almost no longer visible when the test strip is inserted correctly.)

viii. Make sure the code on the meter matches the code on the test strip container.

ix. When you see the flashing blood drop, hold the lancet device against the side of patient fingertip and press the release button.

x. Gently squeeze patient fingertip to get a drop of blood. xi. Once the the blood drop appears on the screen, you have 2 minutes to apply the drop of blood.

xii. Touch the blood drop to the center of the square orange pad. Do not bend the test strip.

xiii. An hourglass symbol appears on the screen, and then the test result appears.

xiv. To remove the lancet, take off the lancet device cap and point the lancet end away.

xv. Slide out the rejector to discharge the lancet into an appropriate container for sharp objects.

xvi. Applying Blood with Test Strip outside of the Meter. xvii. Take the test strip out of the meter

xviii. Touch the center of the square orange pad to the drop of blood. Do not bend the test strip. xix. Within 20 seconds, insert the test strip, with the orange pad to the drop of blood.

### *5.1.13 Biological Reference Intervals*

| | Fasting Blood glucose (mmol/L) | Random Blood Glucose |
|---|---|---|
| Blood/Plasma: | 3.9 – 5.6 mmol/L (70 - 100 mg/dl) | ≤6.9 (125 mg/dl) |

### *5.1.14 Interpretation and reporting of results*

i. Results are displayed in either mg/dl or mmol/liter depending on which unit of measurement is selected. Report the value in the agreed SI unit.

ii. If the result is lower than 10mg/dL (0.6mm/L) "Lo" is displayed instead of a result.

iii. "Lo" may indicate that your blood is very low. iv. If the result is higher than 600mg/dL (33.3mmol/L), "Hi" is displayed instead of a result.

v. Fasting blood glucose between 5.6 – 6.9 mmol/L (100 – 125 mg/dl) indicates high risk to diabtes. vi. Two separate test results of 7.0 mmol/L (126 mg/dl) or higher indicate diagnosis of diabetes.

***Critical value***

| | |
|---|---|
| Fasting blood glucose | <2mmol/L |
| | >20mmol/L |

***5.1.15 Limitation of the Procedure and Sources of Error***

i. Falsely elevated glucose results may be obtained when a person's blood contains bilirubin (unconjugated) >340 µmol/l (>20 mg/dl), triglycerides >57 mmol/l
ii. Abnormal uric acid levels may also interfere with test results. Caution is needd in the interpretation of neoNot applicablete blood glucose values <2.8 mmol/l (<50 mg/dl).
iii. Abnormal haematocrit values may affect test results. iv. Haematocrit levels below 0.20 may cause falsely low glucose values when the glucose concentration is less than or equal to 11.1 mmol/L. Values above 0.55 may cause falsely low glucose values when the glucose is above 11.1 mmol/l.

***5.1.16 Perfomance Characteristics*** Refer to the verification report

***5.1.17 Supporting Document*** Not applicable

***5.1.18 References***

*ACCU-CHEK A*ctive user's manual.

## 5.2 PROCEDURE FOR TESTING BLOOD GLUCOSE BY GLUCO PLUS

***5.2.1 Purpose***

This Standard Operating Procedure (SOP) is aimed to describe step by step on how to operate **Gluco plus device**

***5.2.2 Scope***

This procedure for Gluco-plus device will be used for blood glucose chemistry testing in health facility in Tanzania

***5.2.3 Responsible***

Trained, qualified and competent laboratory registered practitioners are responsible for performing this procedure.

The head of section for chemistry is responsible for ensuring the effective implementation and competency assessment for this procedure .

***5.2.4 Principle*** Not applicable

***5.2.5 Sample Requirements*** Whole blood

***5.2.6 Equipment***

Perform the procedure for start-up, maintenance, troubleshooting and shut down the Gluco-plus as per manufacturer's instrument instructions

### *5.2.7 Materials*

Test strips and Lancing device

### *5.2.8 Storage and Stability*

- Gluco-plus strips should be stored at room temperature
- All reagents should be protected from direct sunlight, extreme heat, and freezing during shipment and storage.
- Temperatures below 32° F (0°C) may cause reagent layering that changes the tonicity and conductivity of the reagents.
- Sample stability after collection of venous whole blood: ✓ Run Samples within one hour of collection.

### *5.2.9 Safety*

- Decontaminate working surfaces twice daily, in the morning and afternoon
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.
- Refer to Manufacturer instruction, National infection prevention and control
- Guidelines for health waste management and safety practice.

***5.2.10 Calibration*** Not Applicable

### *5.2.11 Quality Control*

Control solution

Test prepared GLUCOPLUS control Solution;

i. Once per week
ii. When opening new strips kit
iii. When you suspect the meter or test strips are not working properly. iv. If you drop/damage the meter.
v. Record results on QC Form.

### *5.2.12 Procedural Steps*

| **Step** | **Action** |
|---|---|
| **Changing strip code** | |
| 1 | Check the code on the test strip vial before inserting the test strip. |
| 2 | Insert the test strip to turn ON the meter and match the code on the meter with the code on the strips vial. |
| 3 | If the code is already matched press OK to go to APPLY SAMPLE screen. |
| 4 | If the code in Meter does not match the code on the test strip vial, Press S button until you hear a beep sound. Press **S** or **M** to match the code number on the test strips vial. |
| **Setting time** | |
| 1 | Switch on the analyser |

| | |
|---|---|
| 2 | Press **S** button until you hear a beep sound. Press off button to save the strip code. |
| 3 | Month number will blink use S and M button to select the required month |
| 4 | Press the off button to save month and date number will blink and use S and M button to select the required date. |
| 5 | The time hours will blink use S and M to set time . |
| **Performing a Quality Control test** | |
| 1 | Perform weekly and as per meter (machine) protocol. |
| 2 | Control used is **GLUCOPLUS Selected Control solution**. |
| 3 | Prepare and Apply Control solution. |
| 4 | Touch the sample tip of the test strip to the control drop. Verify check window filled. |
| 5 | Meter display will count down from 5 to 1 and then display result. |
| 6 | Compare the control solution results with correct Control range printed on test strips vial. (Example; 6.2 – 8.2 mmol/L). If the result are not within control range repeat the control solution test. |
| **Testing Blood** | |
| 1 | Use lancing device to puncture site, normally fingertip. |
| 2 | Insert a test strip into the strip port to turn on meter. |
| 3 | Touch the blood sample to the sample tip at the end of the test strip. |
| 4 | The meter display will count down from 5 to 1 and then display result. |
| 5 | If the “HI” or “LO” message appears on the meter, the result is above 33.3 or below 1.1. Repeat test to verify. |
| **Error Codes** | |
| **E-1** | Problem with Meter – Do not use the meter |
| **E-2** | Meter or strip Problem – Repeat the test with New strip. |
| **E-3** | Meter was not ready – Repeat the test with a new strip. Apply SAMPLE or CONTROL appears on the display. |
| **E-4** | Strip Problem – Repeat test with a new strip. |
| **E-5** | Strip problem or Sample too small – Repeat the test with a new strip and new sample. |
| **HI.E** | Temperature too high—repeat test in a cooler area. |
| **LO.E** | Temperature too low—repeat test in a warmer area. |
| **E-6** | Battery LOW — replace battery soon. |

***5.2.13 Biological Reference Interval***

| | Fasting Blood glucose (mmol/L) | Random Blood Glucose |
|---|---|---|
| Blood/Plasma: | 3.9 – 5.6 mmol/L (70 - 100 mg/dl) | ≤6.9 (125 mg/dl) |

***5.2.14 Interpretation and Reporting of Results*** vii. Results are displayed in either mg/dl or mmol/liter depending on which unit of measurement is selected. Report the value in the agreed SI unit.

viii. If the result is lower than 10mg/dL (0.6mm/L) “Lo” is displayed instead of a result.

ix. “Lo” may indicate that your blood is very low.

x. If the result is higher than 600mg/dL (33.3mmol/L), “Hi” is displayed instead of a result.

xi. Fasting blood glucose between 5.6 – 6.9 mmol/L (100 – 125 mg/dl) indicates high risk to diabtes. xii. Two separate test results of 7.0 mmol/L (126 mg/dl) or higher indicate diagnosis of diabetes.

***Critical value***

| | |
|---|---|
| Fasting blood glucose | <2mmol/L |
| | >20mmol/L |

### *5.2.15 Limitation of the Procedure and Sources of Errors*

If the “HI” or “LO” message appears on the meter, the result is above 33.3 or below

### *5.2.16 Perfomance Characteristics*

Refer the method verification reports from for this procedure and equipment manufacturer user manual

### *5.2.17 Supporting document*

Sample Collection Manual, Safety Manual, Quality Manual

### *5.2.18 References*


1. User manual for Gluco-plus
2. Manufacturers package insert


## 5.3 PROCEDURE FOR URIT 50 (URINE CHEMISTRY ANALYZER)

### *5.3.1 Purpose*

This procedure is provides description for performing urine biochemistry by using URIT 50 semi-automated Urine analyser.

### *5.3.2 Scope*

This procedure is applied for testing Urine sample at the health facilities in Tanzania.

### *5.3.3 Responsible*

A trained and competent health laboratory practitioners are responsible for performing this procedure. The head of section of biochemistry and parasitology is responsible of ensuring the implementation of this procedure.

### *5.3.4 Principle*

The analyser measures change of the reflectance of reagent strips pads, A detector integrated in the system is composed of light source and a light receiver, the light from which goes through in the spherical integrator and reflect to the reagent pads

on strip. The absorbance (reflectance) varies according to the color of reagent pads, the darker of the reagent pads higher the absorbance is and less light is reflected. Conversely the lighter the reagent pad is the lower the absorbance is and more light is reflected degree of color developed is direct proportion to the concentration of analyte in urine" .

### *5.3.5 Sample Requirements*

4mls of uncentrifuged mild stream urine sample is used.

### *5.3.6 Equipment*

Urit-50

### *5.3.7 Materials*

Disposable gloves, Laboratory coats, Urine container, Waste container, Marker pen, Urine strips from urit G10, G11 or G14, Gauze

### *5.3.8 Storage and Stability*

i. Store urine sample at room temperature for 30 minutes to 2 hours or 24 **hours** in refrigerator.
ii. Reagent strips and calibrator should be stored to free and clean area at 37°C iii. Control materials should be stored as per the manufacturers instructions

### *5.3.9 Safety*

Samples and control materials at this section should be treated as infectious material and should be handled careful.

### *5.3.10 10.0. Calibration* Not applicable

### *5.3.11 Quality Control*

i. Put on PPE
ii. Install the strip holder into machine
iii. Switch on the machine and wait the machine for initialization
iv. Put the dry or calibrator strip on the strip holder till D sound
v. Wait the machine to scan and print result
vi. Record the QC result.

***Note: Return calibrator strip into its container and discard other used materials according to standard operating procedures***

### *5.3.12 Testing Procedures*

i. Deep the reagent strips of G series into urine sample and put it on the dry gauze to remove excess urine on the back of the strip.
ii. Put the sample strip on the strip holder till D sound
iii. Read the patient result on the machine
iv. Record result to the register

***5.3.13 Biological Reference Intervals*** See annex 3.

***5.3.14 Interpretation And Reporting Of Results***

Refer to the insert which present on the reagents strips of G series G10, G11, G14. Report result according to insert present on the reagent strip bottle

***5.3.15 Limitation of the Procedure and Sources of Errors***

The test relies on correct collection of sample by the patient, and if this is not done properly the results may not be accurate

***5.3.16 Performance Characteristics*** Refer to method verification

***5.3.17 Supporting Documents*** URIT 50 user amnual

***5.3.18 References***

URIT 50 urine user manual.

## 5.4 PROCEDURE FOR (URIT-560) URINE ANALYZER

***5.4.1 Purpose***

This procedure provides instructions for determining urine biochemical test using the URIT-560 analyzer

***5.4.2 Scope***

This procedure is used in Clinical chemistry section of the user when performing urine using the URIT-560 analyzer

***5.4.3 Responsible***

The section head of Clinical Chemistry is responsible for ensuring this procedure is effectively implemented and maintained

***5.4.4 Principle***

The analyzer measures changes in reflectance of the reagent strips pads. A detector integrated in the system is composed of a light source and a light receiver, the light from which goes through spherical integrator and reflect at the reagent pads on the strips. The absorbance varies according to the color of the reagent pads. The darker the reagent pads is the higher the absorbance is and less light is reflected. Conversely, the lighter the reagent pad is the lower the absorbance is, and more light

is reflected; ie. The degree of color development is proportion to the concentration of analyte in urine.

The reflected light goes in to the optical-electronic detector system, which transforms the optical into electrical. The strength of the electricity correlates which reflectance. Then the electrical cables will be processed by CPU after being transformed by I/V converter, and the test results can be printed out by printer.

### *5.4.5 Sample Requirements*

Dry, wide-necked, leak proof container 10-20 ml of urine specimen.

### *5.4.6 Equipment*

URIT-560, Printer

### *5.4.7 Materials*

Urine strips (URIT G Series), Printer thermal paper, Urine container, Gloves

### *5.4.8 Storage and Stability*

Sample is stable for 2hrs at room temperature or 24hrs at 2-8$^0$C

### *5.4.9 Safety*

i. Personnel Protective Equipment must be worn at all times
ii. Samples must be treated as potentially infectious.

### *5.4.10 Calibration*

Perform calibration as per manufacturer instructions

### *5.4.11 Quality Control*

Currently no control

### *5.4.12 Procedural Steps.*

i. Give the patient a sterile, dry, wide-necked, leak proof container and request10-20 ml of urine specimen. ii. Explain to the patient the need to collect the urine with as little contamination as possible i.e. a clean-catch specimen.

iii. On the instrument, press (image test) to enter into the image test interface.
iv. Insert or Pour urine on the test strip, put test strip into test strip holder until the sound of alarm raised.
v. Then wait the printer out results.

### *5.4.13 Biological Reference Intervals.*

| Parameter | Abbreviation | Biological Reference Intervals |
|---|---|---|
| Urobilinogen | URO | Normal |
| Glucose | GLU | Negative |

| | | |
|---|---|---|
| Bilirubin | BIL | Negative |
| Ketones | KET | Negative |
| Specific gravity | S.G | 1.003-1.029 |
| Occult blood | BLD | Negative |
| Ph | Ph | 4.5 - 7.8 |
| Protein | PRO | Negative |
| Nitrite | NIT | Negative |
| Leukocytes | LEU | Negative |

### *5.4.14 Interpretation And Reporting Of Results*

Results are automatically printed from the machine. Attach results printout with report form

### *5.4.15 Limitation of the Procedure and Sources of Errors.*

- Urine must be processed within 2hr to avoid growth of bacteria which consuming glucose and developing ammonia in urine, loose of ketone bodies, Increase of PH
- Urine if not processed on time store in refrigerator $2^{o}C$ – $8^{o}C$ for 24

### *5.4.16 Performance Characteristics*

Refer into method verification report

### *5.4.17 Supporting Documents*

Equipment maintenance form, sample collection manual, quality manual

### *5.4.18 References*

- URIT-560 operator's manual
- Standard guard line for Health Laboratory 2007 Edition.

## 5.5 ROCEDURE FOR PERFORMING URINE BIOCHEMISTRY BY USING CYBOW READER 300

### *5.5.1 Purpose*

This Standard Operating Procedure (SOP) is aimed to describe step by step on how to operate the CYBOW™ **READER 300** semi-automated Urine analyser using Urine sample at the health facilities in Tanzania

### *5.5.2 Scope*

This procedure applies to all staff who works in parasitology section on performing urinalysis test.

### *5.5.3 Responsible*

A trained, qualified and competent laboratory registered practitioner are responsible for performing this procedure. The head of sections is responsible for ensuring the implementation of this procedure.

### *5.5.4 Principle*

The **CYBOW** reader 300 are reflectance photometer. The strip is illuminated by white light, and the reflected light from the strip is detected by the sensor. The RGB signal is digitized, and this digitized image is interpreted by the processor. The intelligent image analyser SW locates the strip and the pads, and based on this colour data the parameter values are determined. The results including the date and the time of measurement, sequence number and the ID are stored printed out by the internal printer.

### *5.5.5 Sample Requirements*

10-20mls of mild stream urine sample collected in sterile wide mouth container is required for performing this test. **Do not** centrifuge urine sample before bio chemical test.

### *5.5.6 Equipment*

Perfom the **CYBOW$^{TM}$ READER 300** procedure for start-up, maintenance, troubleshooting and shut down the urine analyser as per manufacturer's instrument instruction.

### *5.5.7 Materials/REAGENTS*

Gloves, Marker pen, Laboratory coat, Waste container, Gauze, Urine container and CYBOW strip.

### *5.5.8 Storage and Stability*

Sample, reagents, calibrators and control materials should be stored as per the manufacturer instructions.

### *5.5.9 Safety*

- Adhere to safety precautions as stated in the Safety manual
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.
- Front cover of machine should be covered during operation to avoid sample contamination.
- Used only power cord specified for CYBOWTM READER 300
- Avoid excessive dust, wet/damp condition and provide proper ventilation.

- Do not wipe the body clean with benzene, thinner, gasoline. This may discolour

### *5.5.10 Calibration*

The calibration of instrument should be done prior to first time use and then the 2nd calibration process is recommended **in every 4 weeks** with calibration strip provided in the package.

### *5.5.11 Quality Control*

Run internal Quality Control samples daily before examing patient samples to ensure quality of examination results. Other conditions that drive controls include:

After a reagent lot number change
After maintenance, component replacement, or a field service action ✓
After a software change ✓ Following calibration.
According to regulatory requirements

### *5.5.12 Procedural Steps*

- Check if the urine was received within 1 hour of collection and in a sterile universal container
- Mix the urine by swirling the container and dip a CYBOW strip into urine, making sure that the entire measuring region of the strip is immersed then running strip on instrument.

**Mode of running strip on CYBOW™ READER 300**

**1. General mode** • Select the general mode by pressing direction key(◄) and press Enter button to return to the standby mode.

- After the 1st strip dipped in urine and placed on a plate, press start key(►).
- Put 1st-10th (max.) strip on the plate one by one after dipping each in selected urine.
- Once last reagent strip on batch is placed, press Enter button
- After incubation time of the 1st strip, it will start to loading the results of the strip on by one.

2. **One by one mode**
    - Select one by one mode by pressing **Direction** key (◄) and press **Enter** button.
    - After the 1st strip is placed on the loading plate press **start** key (►).
    - Press direction key whenever each of the next strip is placed on the plate one by one.
    - Once last strip is place on strip loading plate, press **Enter** button.
    - After incubation time of the 1ststrip, it start to read and print result of the strip one by one.
3. **Quick mode**

- Select the quick mode by press direction key(◄) and press **Enter** button
- Put the strips (incubation is done) once strip loading plate continuously.
- Once the last strip is placed on the loading plate, press **Enter** button.
- Test result is shown on the LCD and automatically saved in the memory

***5.5.13 Biological Reference Intervals*** See annex 3.

***5.5.14 Interpretation And Reporting Of Results***

***Chemical urinalysis***

Report the reading when the immersed strip is compared to the colours on the strips container

***Macroscopic examination***

Report whether the urine is Clear, Slightly cloudy, Cloudy or turbid. Report the colour of the urine which will range from Light yellow, Yellow, Amber, Red to Brown

***Microscopic examination of urine sediment***

- **White Blood Cells/Pus Cells/ leucocytes.**
  Report the average number of cells per High Power Field, example 2-5 WBCs/HPF
- **Red Blood Cells**
  Report the average number of cells per High Power Field, example 2-5 RBCs/HPF
- **Casts**
  Identify the type of cast and report as number per High Power Field
- **Crystals**
  Identify the type of crystals and report their presence • **Epithelial cells**
  Identify the type whether squamous, transitional or renal epithelial cells, quantify them and report per high power field. Otherwise report the presence of epithelial cells if can't be identified.
- **Trichomonas Vaginalis**
  Report as "seen" or "not seen"
- **Yeast**
  Report as "seen" or "not seen"
- **Spermatozoa**
  Report for males and not for females

***5.5.15 Limitation of the Procedure and Sources of Errors***

Urine samples should be tested within one hour of collection

If any delay happen put the urine sample in the refrigerator to avoid bacterial growth.

Do not use urine dipsticks beyond expiry date

### *5.5.16 Performance Characteristics*

Refer to the method verification report of this procedure

### *5.5.17 Supporting Documents*

Safety manual and Sample collection manual

### *5.5.18 References*

Manufacturer's Package Insert in multistrips kit

Cheesbrough, Monica Health Laboratory Manual for Tropical Countries

Graff's Text Book for Urinalysis and body fluids, Second edition, Lillian A. Mundt and Kristy Shanahan

## 5.6 PROCEDURE FOR DETERMINATION OF ALT BY USING DIRUI-DR 7000 CHEMISTRY ANALYZER

### *5.6.1 Purpose*

This procedure provides instructions for determining Alanine Aminotransferase (ALT) using the DIRUI-DR7000 Analyzer

### *5.6.2 Scope*

This procedure is used in Clinical chemistry section for analysing ALT using the DIRUI-DR7000 clinical chemistry Analyzer.

### *5.6.3 Responsible personnel*

Qualified, trained and competent Health Laboratory Assistant technologists, Technologists, and Scientists are responsible for performing this procedure.. The section head of Clinical Chemistry is responsible for ensuring this procedure is effectively implemented and maintained.

### *5.6.4 Principle*

Kinetic method for the determination of ALT activity according to the recommendations of the Expert Panel of the International Federation of Clinical Chemistry (IFCC). Without pyridoxalphosphate activation. ALT is measured by the reagent rate analysis by the coupled reaction with lactate dehydrogenase (LDH) to reduce NADH (measured at a wavelength of 340nm) to NAD+. The rate of decrease in absorbance at 340 nm due to NADH depletion is proportional to the ALT activity in the sample.

### *5.6.5 Sample requirement*

30µl serum or plasma, sample free from hemolysis.

### *5.6.6 Equipment*

DIRUI DR7000 Semi Automated Chemistry Analyzer, Centrifuge machine

### *5.6.7 Materials*

Reagent Kits, Calibrator/Stsndard, Control Kits (LEVEL I and II), Disposable gloves Laboratory coat, Sample Cups, Reaction Wells, Transfer Pipettes

### *5.6.8 Storage and stability*

Refer to the facility laboratory sample collection manual

### *5.6.9 Safety*

i. Adhere to safety precautions as stated in the facility laboratory Safety manual/IPC guidline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *5.6.10 Calibration*

**i.** Perfom equipment calibration when; - ii. There is a change in the reagent lot number. iii. If the QC result falls outside the acceptable ranges. iv. The machine blinks on the QC-calibration indicating that the calibration is expired.

v. There is a change in the system software
vi. System maintenance/ component replacement procedure is performed

### *5.6.11 Quality control*

QC shluld be performed before patient samples, after calibration of reagent

### *5.6.12 Procedure Steps*

**Running the Calibrator, controls or samples;**

i. Label the tubes for blank, calibrator, Controls or samples
ii. Prepare the working reagents as indicated by the industrial manufacturer
iii. Pipette the working reagents R1 480µl into the labelled tubes
iv. Pipette 30µl of distilled water into the tube labelled blank.
v. Incubate for 300s
vi. Add 120µl of R2 reagent
vii. Incubate for 60s
viii. Read the result

***5.6.13 Biological reference intervals*** See annex 4.

### *5.6.14 Interpretation and Reporting of Results*

**Interpretation of results**

Perfomed results will be displayed on the machine screen

**Result reporting**

Once all of the results are accepted or validated, a final report will automatically be printed out.

### *5.6.15 Limitation of the Procedure and Sources of Error*

Hemolyed, lipemic samples, icterus and anticoagulants such as citrate, oxalate and fluoride (for other tests except Glucose) and drugs such as hydroxocobalamin and Cephalosporin antibiotics.

***5.6.16 Performance Characteristics*** Refer to the verification report

### *5.6.17 Supporting document*

Sample collection manual, quality manual

### *5.6.18 References*

DIRUI-DR7000 Chemistry analyser user manual

## 5.7 PROCEDURE FOR DETERMINATION OF AST BY USING DIRUI-DR7000 CHEMISTRY ANALYZER

### *5.7.1 Purpose*

This procedure provides instructions for determining ASAT using the DIRUI-DR7000 Analyzer

### *5.7.2 Scope*

This procedure is used in Clinical chemistry section of the user when performing blood analysis using the DIRUI-DR7000 clinical chemistry Analyzer

### *5.7.3 Responsible*

The section head of Clinical Chemistry is responsible for ensuring this procedure is effectively implemented and maintained.

### *5.7.4 Principle*

Kinetic method for the determination of Aspartat-Aminotransferase (AST) activity according to the recommendations of the Expert Panel of the International Federation of Clinical Chemistry (IFCC). Without pyridoxalphosphate activation. AST is measured by the reagent rate analysis by the coupled reaction with Malate dehydrogenase (MDH) to reduce NADH (measured at a wavelength of 340nm) to NAD+. The rate of decrease in absorbance at 340 nm due to NADH depletion is proportional to the AST activity in the sample.

### *5.7.5 Sample Requirements*

Serum or plasma, sample free from hemolysis and not contaminated

### *5.7.6 Equipment*

DIRUI DR7000 Semi Automated Chemisry Analyzer•

**Cleaning and Maintenance**

i. Use a large amount of distilled water to rinse the tubing by click the rinse interface.
ii. And drain the liquid from the tubing if necessary. iii. Remove the waste liquid bottle from the back of the analyzer.
iv. Keep the instrument vertical during move and transport.
v. Try best to avoid vibration. vi. And check and debug the instrument before use.

### *5.7.7 Materials*

Reagent Kits, Calibrator/Standard,Control Kits (LEVEL I and II), Supplies, Disposable gloves, Laboratory coat, Sample Cups, Reaction Wells and Transfer Pipettes

### *5.7.8 Storage and Stability*

- Reagent Should be kept at temperature of 2-8°C and sealed in dry place without sunshine. The shelf life is 18 months .
- Under condition of 2-8°C, the open vial stability is 30 days

### *5.7.9 Safety*

Personnel Protective Equipment must be worn at all times and samples must be treated as potentially infectious.

### *5.7.10 Calibration*

It is suggested to use supplementary calibrator as instructed. When lot number is changed or QC is invalid, calibration shall be conducted again. Procedures for reagent, Calibration, QC and Sample preparation

### *5.7.11 Quality Control*

It is suggested to use QC products produced by DIRUI.

### *5.7.12 Procedural steps*

i. Label the tubes for blank, calibrator, Controls or samples
ii. Prepare the working reagents as indicated by the industrial manufacturer
iii. Pipette the working reagents R1 480µl into the labelled tubes
iv. Pipette 30µl of distilled water into the tube labelled blank.
v. Incubate for 300s
vi. Add 120µl of R2 reagent
vii. Incubate for 60s
viii. Read the results

### *5.7.13 Biological Reference Intervals.*

See annex 4.

### *5.7.14 Interpretation and Reporting of Results*

**Reporting of results**

Results are automatically printed from the machine Attach results printout with report form.

***5.7.15 Limitations of the procedure and sources of error.*** Gross hemolysis, Lipemic AND Icterus specimen

### *5.7.16 Performance Characteristics*

Refer verification report

### *5.7.17 Supporting Documents*

Equipment Maintenance Form.

**18.0 References**

DIRUI DR7000 Analyser operator's manual

National standard guard line for Health Laboratory 2007 Edition

## 5.8 PROCEDURE FOR SA-30 SEMI AUTOMATED CHEMISTRY ANALYZER

### *5.8.1 Purpose*

This Procedure describes step by step on how to operate the SA-30 a semiautomated chemistry analyser to perform basic chemistry tests using human serum, plasma or cerebral spinal fluid (CSF) sample.

***5.8.2 Scope*** it is applied in testing biochemistry parameters using human serum or plasma sample in the biochemistry department/section.

### *5.8.3 Responsible*

All qualified, trained and competent laboratory scientist, laboratory technologists and assistant laboratory technologists are responsible for performing this procedure. The head of section of biochemistry is responsible of ensuring the implementation of this procedure.

### *5.8.4 Principle*

The principle of the instrument is based on the phenomenon of different wave band absorbance from substance, which is in line with Lambert-Bill Law.

(The greater the concentration of the sample, the more light is absorbed, the less light is transmitted, and the darker of the color)

### *5.8.5 Sample Requirements*

The 2 - 4ml of whole blood collected in plain tube (red top) for serum or EDTA (purple top) for plasma or in heparinized tube free from hemolysis. Cerebral spinal fluid (CSF) when required.

### *5.8.6 Equipment*

SA-30 semi- automated chemistry analyser, Centrifuge

### *5.8.7 Materials*

Micropipettes and tips, Marker pen, Thermal paper and PPE

### *5.8.8 Storage and Stability*

i. Store serum/plasma at room temperature 25 – 35 °C for 8 hours
ii. Tested sample stored at 2 -8 °C to 7 days. iii. Store reagent,calibrator and controls per manufacture recommendation

### *5.8.9 Safety*

Treat all Samples and control materials as infectious material and should be handled careful as per IPC guidelines

### *5.8.10 Calibration*

Quality Control procedure is the same as analytic procedure of unknown sample (Use Randox Calibrator (pipette 500µl of reagent to tube and add 25µl calibrator mix gently and incubate at $37^0$C for 8-10 minutes for end point test method, no need of extra incubation for kinetics method)

### *5.8.11 Quality Control*

Quality Control procedure is the same as analytic procedure of unknown sample (Use Randox QC ,pipette 500µl of reagent to tube and add 25µl control mix gently and incubate at 37C for 8- 10 minutes end point test method, no need of extra incubation for kinetics test method)

### *5.8.12 Procedural Steps*

i. Prepare for sample and reagent. ii. For End point test method, Add R1 + R2 + Sample in ratio and mix thoroughly and incubate at 37℃ for 8 – 10 Min (refer to reagent user manual)
iii. For kinetics test method, Add R1 + R2 + Sample in ratio and mix thoroughly, no need of extra incubation**.**
iv. For HDL, LDL add R1 + Sample in in ratio, mix them thoroughly incubate at 37℃ in 2 min then add R2 and incubated them in 5-7 Min,
v. Click “Test” on main menu to enters next page to start testing.

vi. Press PUSH button to aspirate distilled water, to calibrate AD value. The AD value should be 45000 to 60000.

vii. Click “Continue” to test reagent blank. viii. Select “YES” to aspirate reagent blank to test reagent blank absorbance.

ix. Press PUSH button to aspirate reagent blank to test reagent blank absorbance.

x. Click “Continue” to test STD

xi. Select “NO”, device will use last factor and perform the sample test directly. Select “YES”, device will aspirate standard to test STD xii. Press PUSH button to aspirate standard, then device will test standard absorbance and calculate factor automatically

xiii. Click “Continue” to next to test the sample directly or perform control test.

xiv. Press PUSH button to aspirate sample or control, then device will test the sample or control and display test result automatically

***5.8.13 Biological Reference Intervals*** See annex 4.

***5.8.14 Interpretation And Reporting Of Results***

**Interpretation**

i. If the result of the particular parameter lies within the established reference range, it means that the patient has normal particular parameter.

ii. If the result of the particular parameter lies below or above the established reference range, it means that the patient has abnormal particular parameter and requires intervention as per the clinical history and the laboratory findings. **Reporting results**

Report the results as they are displayed on the screen of the machine

***5.8.15 Limitation of the Procedure and Sources of Errors***

i. Avoid using the haemolysed and lipemic sample as this will cause falsely elevated values. In this case inform the requesting physician and ask for another specimen.

ii. Avoid exposure of the freshly dissolved substrate to strong sunlight, since the reagent is light sensitive. The change in absorbance will increase with an increase in temperature, since the pH of the reagent will be different at different temperatures

iii. Serum must be separated by centrifugation as soon as possible after collection of the patient's blood sample, preferably <2 hours, otherwise, phosphate present in erythrocytes will be released into the serum causing falsely elevated values

iv. Grossly bloody CSF may give spuriously elevated values. Undue delay in analysis may give low values. The report to the requesting physician should include the appearance of the CSF before and after centrifugation.

***5.8.16 Performance Characteristics*** Refer to method verification report

***5.8.17 Supporting Documents***

Sample collection manual, Safety manual, Quality manual

***5.8.18 References***

SA-30 semi automated chemistry analyser user manual

## 5.9 PROCEDURE FOR OPERATING CLINDIAG FA 200 CHEMISTRY TEST

***5.9.1 Purpose***

This procedure provides instructions for determining basic Chemistry tests using Clindiag FA 200 analyzer.

***5.9.2 Scope***

This procedure is used at Clinical chemistry section for processing basic chemistry tests using Clindiag FA 200 analyzer.

***5.9.3 Responsible***

Qualified and trained Health Laboratory Personnel are responsible for doing this test procedure.

***5.9.4 Principle***

The test principle of the biochemistry analyser is mainly based on the lambert Beer law.

The reagents and the samples to be tested are mixed at a certain proportion. The mixture is placed in a calorimetric dish at a certain temperature for incubation, its absorption of light of specific wavelength is continuously measured, and finally the concentration of the measured substance is automatically calculated according to the value of absorbance (change).The procedure uses **Lambert Beers Law** which state that the absorptive capacity of a dissolved substance is direct proportional to its concentration to the solution.

***5.9.5 Sample Requirements***

Serum is most preferred sample

Refer to the facility Laboratory sample collection manual.

***5.9.6 Equipment***

Clindiag FA200 Chemistry analyser.

***5.9.7 Materials***

Reagent kits, cleaning solution, HD- high efficiency cleaning agent, Sample cups,

Calibrators and controls, Gloves, Laboratory coat, A4 paper, Micropipette, Micropipette tips

### *5.9.8 Storage and Stability*

Samples are stored at 2-8$^{o}$C after testing for 3days.

### *5.9.9 Safety*

i. Adhere to safety precautions as stated in the Safety manual. ii. All personal protective equipment (PPE) must be worn when performing this procedure.

iii. All samples must be regarded as potentially infections. iv. Refer to National infection prevention and control Guidelines for health care.

v. Avoid any contact between hands and eyes and nose during sample collection and testing.

vi. Do not use kit beyond the expiration date. vii. Do not reuse the test device. viii. All spills should be wiped thoroughly using 1% sodium hypochlorite solution.

### *5.9.10 Calibration*

Machine should be calibrated when

- There is a change in the reagent lot number.
- If the QC result falls outside the acceptable ranges.
- There is a change in the system software
- System maintenance/ component replacement procedure is performed

### *5.9.11 Quality Control*

**Follow the following steps to run internal Quality Control.sample** i. Click on task.

ii. Click on add Quality control(QC.)
iii. Click on ADD
iv. Select QC batch number.
v. Select a QC item in QC list
vi. Select type of container
vii. Input the position of QC material on the sample plate
viii. Click on OK
ix. Allow the amchine to perfom test till final results

### *5.9.12 Procedural Steps*

i. Click Task
ii. Click Add sample
iii. Click ADD
iv. Enter patient information
v. Select the test item

vi. Select the sample cup number
vii. Click OK
viii. Click Test on left side ix. Select test sample
x. Then click start test
xi. When sample processing is complete, Select the results from the menu bar, patient result review will display, select name of patient on the left side.
xii. All the undone tests will be shown on the results as NA with a reason on the right side. xiii. Re-running patient sample, repeat the patient order with no change in a sample cup number

***5.9.13 Biological Reference Intervals*** See annex 4.

***5.9.14 Interpretation And Reporting Of Results***

**Results interpretation**

Click on <Results> then <sample Results>. The results appear on the computer screen, from here you can select the desired results and release them.

**Reporting of the results**

To print the results, select the sample interface in browse result and click print the test results. Results. **Critical results**

Critical results should be immediately communicated to the clinicians requested the examination. Refer to the annex …. For more details on critical results for clinical chemistry assays.

***5.9.15 Limitation of the Procedure and Sources of Errors***

Do not proces hemolysed samples as they might lead to falsely high results of potassium and low results for glucose.

Samples for glucose investigation should be processed within 2 hours of collection; any delay would cause falsely low results.

Potential operator errors and clindiag FA 200 system technology limitations.

Communicate the following Panic/Critical values to the clinicians

***5.9.16 Performance Characteristics***

Method verification of this procedure should be done and that the report should be referred to verify compliance to this requirement.

***5.9.17 Supporting Documents***

Sample collection manual

***5.9.18 References***

Refer to equipment instruction manual Clindiag FA 200

## 5.10 PROCEDURE FOR DETERMINATION OF IMMUNOASSAYS BY USING GETEIN 1100 IMMUNOANALYSER

### *5.10.1 Purpose*

This procedure provides description on determination of immunoassays by using Getein 1100 Immunofluorescence Quantitative analyser.

### *5.10.2 Scope*

This procedure is used for processing and analysis of immunol assay tests in the biochemistry department/section at the hospital laboratory.

### *5.10.3 Responsible*

A qualified,trained and competent laboratory scientist, laboratory technologists and assistant laboratory technologists are responsible for performing this procedure. The head of section of biochemistry is responsible of ensuring the implementation of this procedure.

### *5.10.4 Principle*

The detection element scans the binding area and converts the optical signal to electrical signal. The voltage variation between the test line and background has a linear relationship with the antigen concentration which can be used to calculate the concentration. In conclusion the antigen concentration in whole blood, plasma, serum, urine can be calculated quantitatively according to optical signal of the test line.

### *5.10.5 Sample Requirements*

The 2 - 4ml of whole blood collected in plain tube (red top) for serum or EDTA (purple top) for plasma or in heparinized tube free from hemolysis. urine sample will be collected in urine container if required.

### *5.10.6 Equipment*

Getein1100 Immunofluorescence Analyzer, stop watch

### *5.10.7 Materials*

Disposable gloves, Micropipettes and its tips, Containers for waste segregation, Marker pen, Getein test card

### *5.10.8 Storage and Stability*

Store unproceesed samples at room temperature for 12 hours. Store performed samples at 2 - 8°c up to 7 days.

Calibrator, controls and test kit devices should be stored as per manufactures instruction

### *5.10.9 Safety*

i. Samples and control materials should be treated as infectious material ii. Worn PPE all of the time while working

### *5.10.10 Calibration*

Calibration of the assay should be peformed as per manufacturer instruction.

### *5.10.11 Quality Control*

Use commercial available controls or inhouse controls to run QC as per schedule

### *5.10.12 Procedural Steps*

i. Refer to the user manual or material data sheet to specific items including reaction time and sample volume carefully for accurate information
ii. Add patient information including ID, name, age, gender, types of sample and test mode to be used
iii. Click start after inserting the card, test item will be auto-recognized and the result will be shown on the screen. User can also see the voltage waveform by slide to the left side
iv. Normally, the test card will auto-quit after testing if not please click on “Quit” icon to quit manually

### *5.10.13 Biological Reference Intervals* See annex 4.

### *5.10.14 Interpretation And Reporting Of Results*

**Interpretation of results**

If the result of the particular parameter lie within established reference range, it means that the patient has normal particular parameter

If the results of the particular parameter lie below or above established reference range, it means that the patient has abnormal particular parameter and requires intervention as per the clinical history and the laboratory finding**.**

**Reporting of results**

Report the result as they are displayed on the screen of the machine.

### *5.10.15 Limitation of the Procedure and Sources of Errors*

Only used for in vitro analysis of human whole blood, serum, plasma, urine or stool freezed samples can not be used for testing due to loss of enzyme or hormone activities

### *5.10.16 Performance Characteristics*

Reffer to verification report.

### *5.10.17 Supporting Documents*

- Sample collection manual
- Material data sheet or reagent manual
- Safety manual

### *5.10.18 References*

Getein 1100 user manual

## 5.11 PROCEDURE FOR OPERATING FIA 8000 ANALYSER

### *5.11.1 Purpose*

This procedure provides instructions for operating FIA 8000 Quantitative Immunoassay Analyzer for biomarkers.

### *5.11.2 Scope*

This FIA8000 is an analyzer that used to measure biomarkers in human whole blood, serum, plasma or urine samples.

### *5.11.3 Responsible*

Qualified and competent registered Health Laboratory practititioners are responsible for doing this test procedure. The head of section of chemistry is responsible for ensuring the effective implementation and competency assessment for this procedure.

### *5.11.4 Principle*

The combination of the antigensin the sample, the gold-labelantibodyin the colloidal gold pad or nitrocellulose membrane, and theantibody pre-coated on the test linecan form a purplish red streak on the test line. The colour intensity of the test lineis proportionate to the quantity of antigens detectedin the sample. The analysersystem can obtain the photo-electric signal intensity of the complexby scanning the test line with a photo-electric component. Then the voltage difference between the voltage of the test line and the background is obtained. The voltagedifference has a linear relationship with the antigen concentrationwhichcan be used to calculate the antigen concentration. The relationship has been established and varying from the measured parameter. In conclusion, the antigen concentration in whole blood, plasma, serum, urine can be calculated quantitatively in one-step according to the colour intensity of the test line.

***5.11.5 Sample Requirements*** Centrifuged Serum and plasma

### *5.11.6 Equipment*

FIA 8000 Quantitative Immunoassay Analyzer

### *5.11.7 Materials*

Test kit, Power source, Printing paper, QC Card, QC SD, Gloves

### *5.11.8 Storage and Stability*

Fresh sample is preferred however if can not be done sample can be stored at 2-8ºC not more than 3days.

### *5.11.9 Safety*

i. Decontaminate working surfaces twice daily, in the morning and afternoon ii. Adhere to safety precautions as stated in the Safety manual

iii. All personal protective equipment (PPE) must be worn when performing this procedure.
iv. All samples must be regarded as potentially infections.
v. Avoid any contact between hands and eyes and nose during sample collection and testing.
vi. All spills should be wiped thoroughly using 1% sodium hypochlorite solution
vii. Decontaminate the biohazpus waste before disposal.

### *5.11.10 Calibration*

**Perfom equipment calibration when; -**

i. There is a change in the reagent lot number.
ii. If the QC result falls outside the acceptable ranges.
iii. The machine blinks on the QC-calibration indicating that the calibration is expired.
iv. There is a change in the system software
v. System maintenance/ component replacement procedure is performed

### *5.11.11 Quality Control*

Quality Control (QC) card should be run before processing patient sample for each day

### *5.11.12 Procedural Steps*

i. Centrifuge collected whole blood samples to obtain serum or plasma.
ii. Mix urine samples thoroughly before testing.
iii. Allow samples to reach room temperature before testing.
iv. Turn on the analyser and select the test.
v. Touch the screen to turn on the analyzer.
vi. Select the test you want to perform from the list of available tests.
vii. Insert the test card.

viii. Open the test card slot.
ix. Carefully insert the test card into the slot, making sure that the arrows on the test card are pointing in the same direction as the arrows on the analyzer.
x. Close the test card slot.
xi. Add the sample.
xii. Follow the on-screen instructions to add the sample to the test card. Be careful not to overfill the sample well.
xiii. Touch the screen to start the test.
xiv. The analyzer will automatically begin to process the sample.
xv. Read the results.
xvi. The analyzer will automatically read the results and display them on the screen.
xvii. The results may be displayed in a variety of formats, such as quantitative results, qualitative results, or graphs.

***5.11.13 Biological Reference Intervals*** See annex 4.

### *5.11.14 Interpretation And Reporting Of Results*

**Interpretation of results**

Interpretate results based on the Biological reference interval.

Normal results are patient results which fall within the reference range for the particular test. Abnormal results are those that fall below or above the reference range. The test report is labeled H: High and L: Low to show the abnormality obtained.

**Reporting of results**

Report the obtained and displayed results in request form/register

**Critical results**

| **Analyte** | **Less Than** | **Greater Than** |
|---|---|---|
| Amylase | 25U/L | 150U/L |
| Chloride | 85 mmol/L | 115mmol/L |
| CK | 30U/L | 200U/L |
| Creatinine | 26umol/L | 120umol/L |
| Glucose(fasting) | 2.5mmol/L | 20.0mmol/L |
| Potassium | 2.5mmol/L | 6.0mmol/L |
| Sodium | 120mmol/L | 160mmol/L |
| Bilirubin Total | 3.4umol/L | 20.5umol/L |
| Biliribun Total for new Born | **Newborn**<br>24hours ≥ 1374umol/L<br>48hours ≥ 2224umol/L<br>84hours ≥2904umol/L<br>One week to one month ≥3424umol/L | |
| Urea (BUN) | ≤1.0mmol/L ≥ 54mmol/L | |

### *5.11.15 Limitation of the Procedure and Sources of Errors*

Haemolyzed sample should not be used since the color changes caused by haemolysis may result to wrong results. Refer to package insert for interfering substances for specific test

***5.11.16 Performance Characteristics*** Refer to the method verification report.

***5.11.17 Supporting Documents*** Sample collection manual

### *5.11.18 References*

User manual for FIA 8000 Analyser.

## 5.12 PROCEDURE FOR OPERATING ALERE AFFINION AS100ANALYSER

### *5.12.1 Purpose*

The purpose of this procedure is to provide instructions on how to operate ALERE AFFINION AS100 analyser for HbA1c, Lipid panel and C - reactive protein.

### *5.12.2 Scope*

This procedure is used in Clinical chemistry section for processing HbA1c,Lipid panel and C-Reactive Protein.

### *5.12.3 Responsible*

A trained, qualified and competent laboratory registered practitioners are responsible for performing this procedure. The head of section for chemistry is responsible for ensuring the effective implementation and competency assessment for this procedure .

### *5.12.4 Principle*

A Test Cartridge with patient sample or control is placed in the cartridge chamber of the Analyzer. By manually closing the lid, the Test Cartridge is transported into the analysis compartment of the Analyzer. Test and lot-specific information is obtained from the barcode label (Figure 2). When the Test Cartridge enters the Analyzer, the integrated camera reads the barcode. The calibration data for the actual lot are read, which then initiates the processing of the Test Cartridge. The sample and reagents are automatically transferred between the wells. An integrated camera monitors the entire process. Light-emitting diodes (LEDs) illuminate the reaction area, which can be either a colored membrane or a reaction well. The camera detects the reflected or transmitted light, which is converted to a test result and displayed on the touch

screen. When the user accepts the result, the lid covering the cartridge chamber opens automatically and the used Test Cartridge can be removed and discarded. The Analyzer is then ready for the next run.

### *5.12.5 Sample Requirements*

Whole blood/Serum / plasma as specified in the reagents insert or as stated in the sample collection manual.

### *5.12.6 Equipment*

Alere Affinion AS100

### *5.12.7 Materials*

Test cartridge, Calibrators, Controls, PPEs, Pipette tips 100 -200ul, Pipette tips 100, 1000ul, Sample container rack, waste bin

### *5.12.8 Storage and Stability*

- Test cartridge should be stored in refrigerator at 2-8$^{o}$C in sealed foil pouches and only stable until expiration date. If not refrigerated they can be stored at room temperature(15-25$^{o}$C) for four weeks.
- Test cartridges should not be exposed to direct sunlight at relative humidity below 90%.
- Whole blood samples can be stored refrigerate at 2-8$^{o}$ C for 3 days. Plasma and serum samples can be refrigerated for 10 days or frozen up 1 year if the tubes are properly sealed.

### *5.12.9 Safety*

i. Adhere to safety precautions as stated in the Safety manual/IPC guidline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *5.12.10 Calibration*

Perform calibration as per Alere Afinion™ AS100 user manual.

### *5.12.11 Quality Control*

Quality control should be done as prescribed in the quality management procedure. Use commercial or in-house made quality control materials to perform on daily basis before testing patient samples. Commercially available quality materials should be used to verify performance of the procedure at least after 100 patient samples have been tested. Lot to lot verification should also be used to check performance acceptability of reagents.

### *5.12.12 **Procedural Steps*** Analyzing a patient/control sample

| | | |
|---|---|---|
| 1 |  | Touch to enter the patient sample mode.<br>Touch to enter the control mode. A “C” in the upper left corner indicates that the Analyzer is in the control mode. The lid opens automatically.<br>*If the lid is left open from the previous run and “Insert Cartridge” is displayed, this step is omitted and you can start with step 2.* |
| 2 |  | Insert the Test Cartridge with the barcode label facing left.<br>*Be sure that the Test Cartridge is correctly placed in the cartridge chamber.* |
| 3 |  | Close the lid manually. The Analyzer will start processing the Test Cartridge.<br>*The processing time depends on the test in use.* |
| 4 |  | Touch and enter the patient ID.<br>Touch to confirm.<br>Touch and enter the control ID or Alere Afinion™ Control Data<br>Touch to confirm.<br>*Entering the patient ID, control ID or Alere Afinion™ Control Data will not interrupt the processing.* |
| 5 |  | Record the result, then touch ✓ to accept.<br>If a printer is connected, touch to print the result.<br>The lid opens automatically.<br>*The result will be saved in the result records.* |
| 6 |  | Remove the used Test Cartridge from the cartridge chamber and discard it in a suitable waste container. Insert a new Test Cartridge or close the lid manually.<br>*Keep the lid closed to protect the cartridge chamber when the Analyzer is not in use.* |

### *5.12.13 **Biological Refences*** See annex 4.

### *5.12.14 **Interpretation And Reporting Of Results*** **Interpretation of results**

- Interpretation of results is based on the Biological reference interval;
- Normal results are patient results which fall within the reference range for the particular test.
- Abnormal results are those that fall below or above the reference range.

**Reporting of Results**

Report the displayed/printed results into register/request form

### *5.12.15 Limitation of the Procedure and Sources of Error*

i. Icteric samples that appears with yellow colour of the serum or plasma due to bilirubin accumulation.
ii. Samples tested after 24 hours may give unreliable results Avoid using lipemic samples

***5.12.16 Performance Characteristics*** Refer the method verification reports .

### *5.12.17 Supporting Document*

i. Sample Collection Manual, Safety Manual, Quality Manual

### *5.12.18 References*

ALERE AFFINION AS100analyzer user manual

Manufacturer package insert

# CHAPTER SIX: SEROLOGY

## 6.1 PROCEDURE FOR SYPHILIS ANTIBODIES RAPID TEST

### *6.1.1 Purpose*

This procedure provides instructions for Qualitative detection of antibodies of all isotopes against *Treponema pallidum*

### *6.1.2 Scope*

The procedure is used in all Laboratory areas for screening syphilis infection

### *6.1.3 Responsible*

Qualified and trained Medical Laboratory Technicians, Technologists and Scientist are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.1.4 Principle*

The syphilis Ab Rapid test strip (Serum/plasma) is a lateral flow chromatographic immunoassay based on the Principle of the double antigens–sandwich technique, In this test syphilis recombinant antigen is immobilized in the test line region of the strip test device, After sample is added to the sample well of the device it react with syphilis recombinant antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with immobilized syphilis antigens.

If the sample contains syphilis antibodies a coloured line will appear in the test line region indicating positive results. If the sample does not contain syphilis antibodies a coloured line will not appear in the region, indicating a negative result

### *6.1.5 Sample Requirements*

Whole blood/plasma sample in purple tube (EDTA) Serum from clotted blood sample in plain tube**.**

### *6.1.6 Equipment*

Centrifuge, Timer, Micropipette

**Maintenance**

Maintenance of the equipment should be performed as per schedule

### *6.1.7 Materials*

| Reagents | Consumables |
|---|---|
| Syphilis Ab Rapid Test Strips kit<br>Known Positive control,<br>Known Negative control | Marker pen<br>Examination Gloves |

### *6.1.8 Storage and Stability*

- The kit should be stored at 2-30 $^{o}$C until the expiry date printed on the sealed pouch or as instructed by the manufacturer.
- Do not freeze the kit or exposing it over 30$^{0}$C.
- Store Serum and plasma sample at 2-8$^{0}$C for up to 3 days.
- For long term storage, serum/plasma should be kept below -20$^{o}$C

### *6.1.9 Safety*

i. Adhere to safety precautions as stated in the Safety manual/IPC guideline
ii. All personnel protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *6.1.10 Calibration*

Perform calibration of equipment as per calibration schedule

### *6.1.11 Quality Control*

A known Negative and Positive in-house controls once every week and whenever a new kit is opened.

### *6.1.12 Procedural Steps*

i. Bring the test kit and sample to room temperate before use.
ii. Remove the test from its sealed pouch, and use it as soon as possible.
iii. Place the test strip on a clean, dry flat surface
iv. Label the test strip with the Patient ID
v. For serum or plasma specimen;
vi. Hold the dropper vertically and transfer 2 drops of serum or plasma (approximately 60µl) onto the specimen pad of the test strip.
vii. Read test results in 15 minutes. Do not interpret results after 15 minutes. viii. For whole blood specimen; ix. Hold the dropper vertically and transfer 2 drops of whole blood (approximately 50ul) onto the specimen pad of the test strip.
x. Then add 1 drop of buffer (approximately 30ul) and start the timer.
xi. Read test results in 15 minutes. xii. Do not interpret test results after 15 minutes

***6.1.13 Biological Reference Interval*** Not applicable

***6.1.14 Interpretation and Reporting of Results*** Interpretation of results

**Negative** - Only one coloured band appears on the control(C) region. No apparent band on the test (T) region

**Positive** - In addition to a pink coloured control (C) band, a distinct pink coloured band will also appear in the tests (T) region

**Invalid** – a total absence of colour in both regions or no coloured line appears on the control (C) region is an indication of procedure error or the tests deterioration. Repeat the test with a new kit. **Reporting of results**

Report results as: Syphilis - Negative or syphilis – Positive **Critical value**

Not applicable

### *6.1.15 Limitation of the Procedure and Sources of Error*

i. The syphilis Ab rapid test strip should be stored at room temperature (15-30°c) ii. Humidity and temperature can adversely affect results. iii. Do not use test if pouch is damaged or broken
iv. Do not use it beyond expiration date.
v. Do not perform the test in a room with strong air flow. i.e. an electric fan strong air-condition
vi. Test is for single use only. Do not re use test.

### *6.1.16 Performance Characteristics*

Refer manufacture kit insert for specificity and sensitivity.

### *6.1.17 Supporting Document*

Sample collection manual, Safety manual

### *6.1.18 References*

Manufacturer Kit insert for syphilis

## 6.2 PROCEDURE FOR (HIV) TESTING BY USING BIOLINETM HIV 1/2 3.0 TEST

### *6.2.1 Purpose*

The purpose of this procedure is to describe the method of performing Bio line HIV1/HIV-2 rapid test assay.

### *6.2.2* Scope

This procedure is applicable to all HIV-1/HIV-2 rapid test using Bio line HIV-1/HIV-2

### *6.2.3 Responsible*

Qualified, registered, licenced and trained Medical personnel are responsible for implementing this test procedure.

The Head of Serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.2.4 Principle*

SD Bioline HIV-1/HIV-2 is a rapid HIV qualitative immune-chromatographic assay used to detect antibodies to HIV in human blood, serum or plasma as the sample had been added to sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the sample window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the client window, forming a red line at the client window site. If antibodies to HIV-1 and/or HIV-2 are absent, the antigen selenium colloid flow past the client window and no red line is formed at the client window site.

### *6.2.5 Sample Requirements*

2-3mls Whole blood/plasma/ serum

### *6.2.6 Equipment*

Timer, Centrifuge, Micropipette, Refrigerator.

**Maintenance**

Maintenance of equipment should be performed as per schedule

### *6.2.7 Materials*

Abbott Bioline TM HIV 1/2 3.0 Test/kit, Assay diluent, Disposable gloves, Laboratory coat

### *6.2.8 Storage and Stability*

The test kit should be stored at a temperature between 1$^{o}$C and 30$^{o}$C or as per manufacturer claims

**Whole blood;** If the blood sample is not immediately tested, it should be refrigerated at 2-8$^{0}$C for 3days

**Plasma or serum;** If plasma or serum sample is not tested immediately, it should be refrigerated at 2-8$^{0}$C for 7 days

For storage period longer than 2week, freezing below -20$^{0}$C is required. They should be brought to room temperature 15-30$^{0}$C prior to use.

### *6.2.9 Safety*

Adhere to safety precautions as stated in the facility Safety manual /IPC guideline

All personal protective equipment (PPE) must be worn when performing this procedure.

All samples must be regarded as potentially infectious.

### *6.2.10 Calibration*

Perform calibration of equipment as per calibration schedule

### *6.2.11 Quality Control*

Run known Negative and Positive in-house controls daily before performing patient samples or when new test kit is opened.

### *6.2.12 Procedure Steps*

i. Bring reagents and samples to room temperature before use. ii. Tear off the desired number of test strips from the 10-test card by bending and tearing off along the perforated line.

iii. Label the strips with sample identification number or patient/client identification number.

iv. Peel the foil cover from the reagent area of the test strips.

**For serum or plasma samples;**

v. Apply 10 µl of sample using a precision pipette to the sample pad (marked by the arrow symbol).

vi. In the absence of precision pipette apply 1 drop of sample using plastic Pasteur pipette provided by manufacture in the kit.

vii. Then apply 4 drop of buffer to the sample pad.

viii. Wait for a 10 to 20 minutes and read results.

**For whole blood collected by finger prick method;** ix. Apply 20 µl of sample (collected by EDTA capillary tube) to the sample pad (marked by the arrow symbol).

x. In the absence of precision pipette or EDTA capillary tube, apply 1 drop of sample using plastic Pasteur pipette provided by manufacture in the kit.

xi. then apply 4 drops of buffer to the sample pad. Wait for 10 to 20 minutes and read results.

**For whole blood collected by venepuncture method;** xii. Apply 20 µl of sample using a precision pipette to the sample pad (marked by the arrow symbol).

xiii. Then apply four (4) drops of buffer to the sample pad.

xiv. Wait for 10 minutes (up to 20 minutes) and read results.

### *6.2.13 Biological Reference Interval*

Not applicable

### *6.2.14 Interpretation and Reporting of Results*

**Result interpretation**

**Negative Result**

The presence of only control line(C) within the result window indicate a negative result

**Positive Result**

The presence of two lines as C and T -1(1) within the window indicates positive results for HIV-1

The presence of two lines as C and T -2 (2) within the window indicates positive results for HIV-2

The presence of three lines as C, T-1(1) and T-2(2) within the result window indicates a positive result for HIV-1 and/or HIV -2

**Invalid results**

No presence of control line (C) or/and pink/purple band observed in the result window Indicate an invalid result. The direction may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be retested.

**Reporting of results**

i. Reactive test Results will be reported as POSITIVE
ii. Non-reactive test results will be reported as NEGATIVE

**Critical value**

Not applicable

### *6.2.15 Limitation of the Procedure and Sources of Error*

- Avoid haemolysed sample and beware of lipemic samples
- Samples other than blood have not been validated to give accurate results.
- Intensity of the patient bar does not necessarily correlate to the titre of antibody
- A negative result with BIOLINE HIV-1/2 does not exclude the possibility of an infection with HIV.

### *6.2.16 Performance Characteristics*

Refer to the method verification report of this procedure.

### *6.2.17 Supporting Documents*

Sample collection Manual, HIV rapid testing algorithm

### *6.2.18 References*

Package insert Abbott Bioline ™ HIV-1/2 3.0

## 6.3 PROCEDURE FOR PERFORMING (HIV) BY USING UNIGOLD TEST

### *6.3.1 Purpose*

The purpose of this procedure is to describe the method of testing HIV-1and HIV-2 using Trinity Biotech Uni-Gold test assay.

### *6.3.2 Scope*

This procedure is applicable in all sites that perform Trinity Biotech Uni-Gold HIV test

### *6.3.3 Responsible*

Qualified, registered, licenced and trained Medical personnel are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.3.4 Principle*

Recombinant proteins representing the immune-dominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp-41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. Antibodies to HIV-1 and HIV-2 react with the colloidal gold linked antigens. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

### *6.3.5 Sample Requirements*

Whole blood/plasma sample collected in purple tube (EDTA)

Serum from clotted blood sample in plain tube

Centrifuge sample at 3000rpm for 5 minutes to obtain serum of plasma.

### *6.3.6 Equipment*

Stop watch, Micropipette, Centrifuge, refrigerator

### *6.3.7 Materials*

Uni-Gold$^{TM}$ HIV test-kit, Disposable gloves, Laboratory coat, 70% alcohol

### *6.3.8 Storage and stability*

Uni-Gold$^{TM}$ HIV test device and wash solution should be stored between 2-27$^{0}$C or as per manufacturer instructions

Whole blood specimen should be stores at 2-8$^{0}$C for up to 3 days at -20$^{0}$C or below

### *6.3.9 Safety*

- Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline
- All personal protective equipment (PPE) must be worn when performing this procedure.
- All samples must be regarded as potentially infections.

### *6.3.10 Calibration*

Perform equipment calibration as per Schedule

### *6.3.11 Quality Control*

Run known Negative and Positive in-house controls once a week.

The test strips contain a control line, which turns colored if the run is valid.

### *6.3.12 Procedure Steps*

i. Bring reagents and samples to room temperature at least 20 minutes before use.
ii. Remove the test device from its protective wrapper.
iii. Label the device with sample identification number or patient/client identification number.
iv. Peel the foil cover from the reagent area of the test strips.
v. For serum or plasma or whole blood collected by finger prick or venipuncture samples;
vi. Using one of the disposable pipettes supplied with the kit, fill it with the sample.
vii. Holding the pipette over the sample port, add two drops of sample (approximately 60 µl) carefully to the sample port of the test device.
viii. Add two drops (approximately 60 µl) of wash reagent to sample port and start the timer.
ix. Wait for a minimum of 10 minutes (up to 12 minutes) and read results

### *6.3.13 Biological Reference Intervals*

Not Applicable

### *6.3.14 Interpretation and Reporting of Results*

**Results interpretation Reactive test results**

Two pink/red lines of the intensity in the device window, the first adjacent to letter ‘T’ (test) and the second adjacent to ‘C’ (control). **Non – reactive test results**

A pink/red line of the intensity adjacent to the letter ‘C’ (control). But no pink/red line adjacent to ‘T’ (test) this indicates a Non-Reactive result. **Invalid results**

No pink/red line appears in the device window adjacent to the letter “C” (control) irrespective of weather or not a pink /red line appears in the device window adjacent to “T” (test). This is an **INVALID** result that cannot be interpreted. An invalid result must be repeated

**Results reporting**

- **Reactive test results:** Report as **HIV TEST POSITIVE**
- **Non-reactive test results-**repeat 1st and 2nd tests following the national HIV testing algorithm
- If the test results are still discordant report - **INCONCLUSIVE** then inform the patient for retesting after 14 days
- If after 14 days, the test is still discordant report - **INCONCLUSIVE**

Collect fresh venous blood sample, refer the sample for ELISA testing

**Critical Values** Not Applicable

### *6.3.15 Limitation of the Procedure and Sources of Error*

i. Avoid hemolyzed sample and beware of lipemic samples when interpreting results.
ii. The BIOLINE HIV-1/2 test is designed to detect antibodies to HIV-1 and HIV-2 in human serum, plasma and whole blood. Other body fluids or pooled samples may not give accurate results.
iii. Intensity of the patient bar does not necessarily correlate to the titer of antibody in the sample. iv. A negative result with BIOLINE HIV-1/2 does not exclude the possibility of an infection with HIV.

### *6.3.16 Performance Characteristics*

Refer to the method verification report

### *6.3.17 Supporting Documents*

Sample collection manual

HIV rapid testing algorithm

### *6.3.18 References*

Manufacture package insert (Trinity Biotech Uni-Gold HIV)

## 6.4 PROCEDURE FOR URINE PREGNANCY TEST

### *6.4.1 Purpose*

This procedure provides instructions for Qualitative detection of HCG in urine

### *6.4.2 Scope*

The procedure is used in the serology section in detection of pregNot applicablency

### *6.4.3 Responsible*

Qualified,trained and competent Medical Laboratory Technicians, Technologists and scientist are responsible for implementing this test procedure.

The Head Microbiology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.4.4 Principle*

The Human Chorionic GoNot applicabledotropin One Step PregNot applicablency Test Strip (urine) is rapid chromatographic immunoassay for the qualitative detection of Human Chorionic GoNot applicabledotropin in urine to aid in early detection of pregNot applicablency. The test uses two lines to indicate results. The test line is pre coated with a monocloNot applicablel Human Chorionic GoNot applicabledotropin antibody to selectively detect elevated level of Human Chorionic GoNot applicabledotropin. The control line is pre coated with goat anti-mouse IgG antibody. The test also includes a burgundy coloured conjugate paid containing another monocloNot applicablel HCG antibody conjugated with colloidal gold. The assay is conducted by immersing the test strip in a urine specimen and observing the formation of coloured lines .The specimen migrate via capillary action along the membrane to reach with coloured conjugate. Positive specimen reacts with the specific antibody HCG coloured conjugate to form a coloured line at the test line region of the membrane. Absence of this coloured line suggest a negative results

### *6.4.5 Sample requirements*

Fresh Urine collected from either morning,evining or any other time

### *6.4.6 Equipment*

Stop watch

Refrigerator

### *6.4.7 Materials*

| Reagents | Consumables |
|---|---|
| HCG test kit | Disposable gloves, Laboratory coat |

### *6.4.8 Storage and stability*

Test strips reagent are stable at 2 to 30$^{o}$ C up to expiration date or as per manufacturer instruction

If sample canot be tested within 1 hour of collection, it should be stored at 2 - 8$^{o}$C for 24hrs

### *6.4.9 Safety*

i. Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline. ii. All personal protective equipment (PPE) must be worn when performing this procedure.

iii. All samples must be regarded as potentially infections.

### *6.4.10 Calibration*

Perform equipment calibration as per schedule

### *6.4.11 Quality control*

Run known Negative and Positive in-house controls (known patient or EQA sample) once a week or when the new kit is opened

### *6.4.12 Procedure Steps*

i. Remove the test strip from the sealed pouch and use it as soon as possible.
ii. With arrow pointing towards the urine specimen immerse the test strip vertically in the urine specimen for at least 10 to 15 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip.
iii. Place the test strip(s) on the non absorbent flat surface, start the timer and wait for the colored line(s) to appear. The results should be ready in 5 minutes.

### *6.4.13 Biological reference intervals*

Not applicable

### *6.4.14 Interpretation and reporting of results*

**Results interpretation**

POSITIVE: two distinct colored lines appear. One line should be the control line region (C) and another line should be on test line region (T).

NEGATIVE: one colored line appears in control line region (C) no apparent colored line appears in the first line (T).

INVALID: control line fails to appear in both the control region. **Reporting of results**

Report results as PregNot applicablency test Negative or PregNot applicablency test Positive.

### *6.4.15 Limitations of the Procedure and Sources of Error*

i. The hCG one step pregnancy test strip (urine) is preliminary qualitative test therefore neither the quantitative nor the rate of increase of hCG can be determined by this test.

ii. Very dilute urine specimen as indicated by low specific gravity may not contain representative level of hCG. If pregnancy is still suspected the first morning urine specimen should be collect 48 hours later and tested.

iii. first trimester pregnancies terminate for natural reasons, a test result that is weakly positive should be confirmed by retesting with first morning urine specimen collected 48 hours later.

iv. This test may produce false positive results. A number of conditions other than pregnancy including trophoblastic disease and certain non trophoblastic neoplasms including testicular tumours, prostate cancer, breast cancer and lung cancer, causes elevated level of hCG. Therefore the presence of hCG in the urine should not be used for the diagnosis pregnancy unless this condition has been ruled out.

v. This test may produce false negative results. False negative results may occur when the levels of hCG are below the levels of sensitivity level of the test. When pregnancy is still suspected a first morning urine specimen should be collected 48 hours later and tested. In case pregnancy is suspected and the test continue to produce negative result see a physician for further diagnosis.

#### *6.4.16 Performance Characteristics*

Refer to the method verification report of this procedure

#### *6.4.17 Supporting documents*

Sample collection manual

#### *6.4.18 References*

HCG package insert

## 6.5 PROCEDURE FOR HEPATITIS C ANTIBODY RAPID TEST

#### *6.5.1 Purpose*

The purpose of this procedure is to give instructions on how to perform Hepatitis C Virus Antibody (HCV Ab) rapid test.

#### *6.5.2 Scope*

This procedure is applicable to all site perform hepatitis C Virus Antibody rapid tests

#### *6.5.3 Responsible*

It is the responsibility of the Head of serology Section to ensure effectively implemented by all personnel working in the serology section.

#### *6.5.4 Principle*

The HCV Ab Rapid test Strip is a lateral flow chromatographic immunoassay based on the Principle of the double antigen- sandwich technique. The test strip consists of

1) a burgundy colored conjugate pad containing HCV antigens conjugated with colloidal gold (HCV Ag conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip contain a test band (T band) and a control band (C band). The T band is pre-coated with non- conjugated HCV antigens, and the C band is pre-coated with goat anti-rabbit IgG. When an adequate of test specimen is dispensed into the sample well of the strip, the specimen migrates by capillary action across the strip. The antibodies: either the IgG, the IgM, or the IgA, to HCV if present in the specimen will bind to the HCV Ag conjugates. The immunocomplex is then captured on the membrane by the pre coated HCV antigens, forming a burgundy colored T band, indicating a HCV Ab positive test result. Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG / rabbit IgG-gold conjugates regardless the presence of any antibodies to HCV. Otherwise, the test result is invalid and the specimen must be retested with another device.

### *6.5.5 Sample requirement*

2-3mls whole blood/serum/plasma. To obtain serum, Centrifuge blood collected in plain red top tube at 3000rpm per 3 minutes. To obtain Plasma, Centrifuge blood collected in EDTA tube at 3000rpm per 3 minutes

### *6.5.6 Equipment*

Timer, Centrifuge and Refrigerator

### *6.5.7 Materials*

| Reagents | Consumables |
|---|---|
| HCV Ab test kit (Test strips, disposable dropper and HCV Ab buffer) | Disposable gloves,<br>Laboratory coat<br>Micropipette |

### *6.5.8 Storage and stability*

a. The test device in the sealed pouch can be stored at 2-40$^0$C or as instructed by manufacturer up to the expiration date. The test device must remain in the sealed pouch until use. DO NOT FREEZE
b. Store whole blood at 2-8$^0$c for up 3 days
c. Serum and plasma maybe stored at 2-8$^0$c for up 7 days, for long term storage, serum and plasma specimens should be kept at-20$^0$c or below.

### *6.5.9 Safety*

i. Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline

ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *6.5.10 Calibration*

Listed equipment will be calibrated as per calibration schedule.

### *6.5.11 Quality control*

Run known Negative and Positive in-house controls once a week or when new test kit is opened

### *6.5.12 Procedure Steps*

Allow test strip, specimen, buffer and/or controls to equilibrate to room temperature prior to testing.

i. Remove the test device from the foil pouch and use it as soon as possible. best results will be obtained if the assay is performed within one hour.
ii. Place the test device on a clean and level surface.
iii. Label the test device with sample ID
iv. **For venepuncture** whole blood samples; Hold the dropper vertically and transfer 2 drops of venipuncture whole blood (approximately 50ul) to the sample pad of the strip, then add 1 drop of buffer (approximately30ul) and start the timer.

**For finger stick** whole blood sample; allow 2 hanging drops of fingerstick whole blood (approximately 50ul) to fall into the center of the sample pad on the test strip, then add 1 drop of buffer(approximately 30ul) and start the timer.

**For serum or plasma sample**; Hold the dropper vertically and transfer 1 drop of serum or plasma(approximately30ul) to the sample pad of the test strip, then add 1 drop of buffer (approximately 30ul) and start the timer.

v. Wait for the red line(s)to appear. the result should be read in 15 minutes.do not interpret the result after 15 minutes

### *6.5.13 Biological Reference Intervals*

Not Applicable

### *6.5.14 Interpretation and Reporting of Results* **Interpretation of results**

**Positive**; two colored lines should be observed. The line in the test region (T) is the prone line; The line in the control region (C) is the control line, which is used to indicate proper performance of the device.

**Negative**; The control line appears in the test, but the test line is not visible.

**Invalid**; No line appears in the control region. Under no circumstances should a positive sample be identified until the control line forms in the viewing area if the control line does not form, the test result is inconclusive and the assay should be repeated

**Reporting of results**
**Reactive test result** - HCV Ab rapid test **positive**
**Non - reactive test results** - HCV Ab rapid test **Negative**

### *6.5.15 Limitations Of The Procedure And Source Of Error*

i. The HCV Ab Rapid Test cassette (whole blood, serum, plasma) is for in vitro diagnostic use only. This test should be used for the detection of antibodies to HCV in whole blood, serum or plasma sample.
ii. The HCV Ab Rapid Test cassette (whole blood, serum, plasma) will only indicate the presence of antibodies to HCV in the sample and should not be used as the sole criteria for the diagnosis of hepatitis C viral infection. iii. A negative result can occur if the quantity of the antibodies to HCV present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.

### *6.5.16 Performance Characteristics*

Refer to method verification report of this procedure

### *6.5.17 Supporting Document*

Sample collection manual

### *6.5.18 References*

HCV Ab Package inserts

Kit manufacturer paper insert

## 6.6 PROCEDURE FOR CRYPTOCOCCAL ANTIGEN RAPID TEST PROCEDURE

### *6.6.1 Purpose*

The purpose of this procedure is to give instructions on how to perform cryptococcal antigen rapid test.

### *6.6.2 Scope*

This procedure will be used by all staff and students perform CrAg test

### *6.6.3 Responsible*

It is the responsibility of the Head of serology Section to ensure effectively implemented and maintained.

### *6.6.4 Principle*

The CrAg Lateral Flow Assay is a dipstick sandwich immune-chromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold- conjugated, anti-Crag monocloNot applicablel antibodies and gold conjugated control antibodies deposited on the test membrane. If Crag is present in the specimen, then it binds to the gold conjugated, anti-CrAg. The gold labelled antibody antigen complex continues to pick up the membrane where it will interact with the test line, which has immobilized ant Crag monocloNot applicablel antibodies. The gold labelled antibody-antigen complex forms a sandwich at the test line causing a visible line o form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold- conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results from only one line (control). If control line fails to develop then the test is invalid.

### *6.6.5 Sample Requirement*

2-3mls serum, plasma, whole blood (venous and finger prick) and cerebral spinal fluid;

**NOTE 1:** To obtain serum, Centrifuge blood collected in plain red top tube at 3000rpm per 3 minutes

**NOTE 2:** To obtain Plasma, Centrifuge blood collected in EDTA tube at 3000rpm per 3 minutes

### *6.6.6 Equipment*

Timer, Centrifuge and Refrigerator

### *6.6.7 Materials*

| Reagents | Consumables |
|---|---|
| CrAg test kit (CrAg LF Test strip, LF Specimen diluents, LF titration diluents, CrAg positive control) | Disposable gloves,<br>Laboratory coat<br>Micropipette |

### *6.6.8 Storage And Stability*

i. If a delay is encountered in sample processing, store at 2-8ºc for up to 72 hours is permissible.
ii. CSF, plasma, serum may be stored for longer period at -20ºc provided they are not repeatedly thawed and refrozen
iii. Whole blood in transit should be maintained at 2-8ºc not -20ºc.

### *6.6.9 Safety*

i. Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *6.6.10 Calibration*

Perform Equipment calibration as scheduled.

### *6.6.11 Quality Control*

i. To ensure assay validity, a procedural control bar is incorporated in the assay device.
ii. Run known positive and negative in-house controls weekly and when the new kit of test strips is opened to verify kit.

### *6.6.12 Procedure Steps*

i. Add one drop /40µ pipette LF Sample diluents to an appropriate labeled reservoir. (Test tube) it is also a good practice to label the strip. ii. Add 40µ of sample to the reservoir and mix. iii. Submerge the white end of a CrAg LF test strip into the sample.

iv. Wait 10 minutes after inserting the strip
v. Read and record the results

### *6.6.13 Biological Reference Intervals*

Not applicable

### *6.6.14 Interpretation And Reporting Of Results*

**Interpretation of results**
**Positive Results**
Two red bands appear on the membrane. One band appear on the control region (C) and another band appears on the test region (T) **Negative Results**

Only one red band appears on the control region C. No apparent red band appears in the test region T.

**Invalid Results**

No visible band at all or there is visible band only in the test region and not in the control region, Repeat the procedure.

**Reporting of results**

For the positive result report **as Cryptococcal antigen rapid test Positive**
For the Negative result report **as Cryptococcal antigen rapid test Negative**

### *6.6.15 Limitations Of The Procedure And Source Of Error*

- **i.** The assay Performance Characteristics have not been established for matrices other than serum, plasma, whole blood and CSF
- **ii.** Depending on the disease and organism prevalence, testing should not be performed as screening procedure for general population. The predictive value of a positive or negative serologic result depends on the pre-test likelihood of cryptococcal disease being present. Testing should only be done when clinical evidence suggests the diagnosis of cryptococcal disease
- **iii.** Testing hemolyzed serum samples could lead false negatives due to the high background color on the strip
- **iv.** This assay was not evaluated for potential interference related to specimen pre-treatment with 2-mercatoethanol or with specimens including the following substances: Vaginal cream, caffeine, ascorbic acid, intraconazole, amphotericin B, acetaminophen, or acetylsalicylic acid

### *6.6.16 Performance Characteristics*

Refer to method verification report of this procedure

### *6.6.17 Supporting Document*

Sample collection manual

### *6.6.18 References*

## 6.7 PROCEDURE FOR PERFORMING (HBsAg) RAPID TEST

### *6.7.1 Purpose*

The purpose of this procedure is to give instructions on how to perform Hepatitis B Virus Surface Antigen Rapid test in human whole blood, plasma or serum.

### *6.7.2 Scope*

This procedure is applicable in all sites that perform Hepatitis B Virus surface antigen in whole blood ,serum or plasma sample qualitatively.

### *6.7.3 Responsible*

It is the responsibility of the Head of serology section and all laboratory personnel to ensure effective implementation of this procedure.

### *6.7.4 Principle*

HBsAg is an antibody sandwich immunoassay. Colloidal gold conjugated monoclonal antibody reactive to HBsAg is dry-immobilized onto a nitrocellulose membrane strip. When the sample is added, it migrates by capillary diffusion through the strip rehydrating the gold conjugate. If present, HBsAg will bind with the gold conjugate antibody to form particles. These particles will continue to migrate along the strip until the test zone (T) where they are captured by ant-HBs antibody immobilized there and a visible red line appears. If there is no HBsAg in sample, no red line will appear in the T zone. The gold conjugate will continue to migrate alone until is captured in the control zone (C) by immobilized goat, ant-mouse IgG antibody aggregating a red line, to serve as an internal process control, a control band should always be seen after test is completed. Absence of a colored control line in the control region is an indication of an invalid result.

### *6.7.5 Sample requirements*

2-3mls Whole blood, centrifuged Serum or plasma samples

### *6.7.6 Equipment*

i. Timer
ii. Refrigerator
iii. Centrifuge

### *6.7.7 Materials*

| Reagents | Consumables |
|---|---|
| HBsAg test kits (HBsAg Device, disposable specimen droppers)<br>Assay diluent | Disposable gloves,<br>Laboratory coat<br>Micropipette |

### *6.7.8 Storage And Stability*

a. The test device in the sealed pouch can be stored at 2-40$^0$C or as instructed by manufacturer up to the expiration date. The test device must remain in the sealed pouch until use.DO NOT FREEZE.
b. Store whole blood at 2-8$^0$c for up 3 days
c. Serum and plasma maybe stored at 2-8$^0$c for up 7 days, for long term storage, serum and plasma specimens should be kept at-20$^0$c or below.

### *6.7.9 Safety*

i. Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.

iii. All samples must be regarded as potentially infections.

### *6.7.10 Calibration*

Perform Equipment calibration as scheduled.

### *6.7.11 Quality Control*

Run known Negative and Positive in-house controls once a week or when new test kit is opened .

### *6.7.12 Procedure Steps*

i. Remove the test strip from foil pouch and use as soon as possible. **Note: Check and verify the device's integrity before and after opening the foil pouch**
ii. Label the test device with patient ID **Whole blood specimen:**
iii. Hold the dropper vertically and transfer 2 drops of whole blood (approximately 50-60 ul) to the specimen area, then add one drop of buffer

**Serum/ plasma;**

i. Immerse the strip into the sample tube with the arrow end pointing towards the sample.
ii. Let it stay immersed until you see liquid traveling up past the MAX word.
iii. Lay the strip (MAX side facing up) flat on a clean, dry, non-absorbent surface
iv. If cassette; Add 60µl (2 drop) of serum or plasma in to the sample window and allow to soak in.
v. Read the results at 15-20 minutes. Ensure that the background of the test area is white before interpreting the results

### *6.7.13 Biological Reference Intervals*

Not Applicable

### *6.7.14 Interpretation And Reporting Of Results*

**Interpretation Of Results**

**NEGATIVE:** if only one line (control line) appears in result line area, interpret the result as negative. This shows that the concentration of HBsAg in the sample is under the detection limit.

**POSITIVE**: if only two line (control line and test) appears in result line area, interpret the result as positive.

**INVALID:** Control line fails to appear. Insufficient sample volume or incorrect procedural technique is the most likely reasons for control line failure. Review the procedure and repeat the test with a new test strip.

**Reporting of results**

Reactive test Results. Report as HBsAg rapid test positive

Non-reactive test results: Report as HBsAg rapid test negative

### *6.7.15 Limitations Of Procedure And Source Of Error*

i. HBsAg Rapid Test Kit detects HBsAg in human serum or plasma and is only screening test. All reactive samples should be confirmed by supplemental assays like PCR OR ELISA.
ii. A non -reactive result does not exclude the possibility of exposure or infection with Hepatitis B virus.
iii. Patients with auto-immune liver diseases may show falsely reactive results.
iv. This test is standardized to work best when the test procedure mentioned in the package insert is strictly followed. Any deviation from the test procedure may lead to erroneous results.

### *6.7.16 Performance Characteristics*

Refer to method verification report of this procedure

### *6.7.17 Supporting Documents*

Sample collection manual

### *6.7.18 References*

HBsAg Package insert kit

## 6.8 PROCEDURE FOR SARS-COV-2 ANTIGEN RAPID DIAGNOSTIC TEST

### *6.8.1 Purpose*

The purpose of this standard operating procedure (SOP) is to provide guidelines to be followed for performing Rapid Antigen Detection Test for COVID-19 using the Standard COVID-19 Ag detection assay kit

### *6.8.2 Scope*

This procedure is to be performed at point of care or any health facility

### *6.8.3 Responsible*

The Head of Serology is responsible for ensuring the effective implementation and maintenance of this procedure

Qualified, competent and registered Medical Laboratory practitioners are responsible for implementing this test procedure.

### *6.8.4 Principle*

It is a rapid chromatographic immunoassay for qualitative detection of specific antigens to SARS-CoV-2. When the liquid sample is dropped on the sample pad, the antigen in the sample forms an immunocomplex with the antibody labelled with colloidal gold. Its complex moves along with the liquid sample, and makes a contact

with the antibody immobilized on the membrane, followed by forming an immunocomplex with the immobilized antibody, resulting in generation of a coloured red purple line. Appearance of red purple line on the membrane indicates the presence of antigen in the sample. Since the liquid of the sample migrates through the membrane very fast, it makes it possible to detect the presence or absence of antigen within 15 minutes.

### *6.8.5 Sample Requirements*

Nasopharyngeal swab sample collected from nostril of the suspect individual. Oropharyngeal swab sample collected from the posterior pharynx and tonsillar area of the suspect individual.

### *6.8.6 Equipment*

Stop watch, Micropipette/Supplied capillary

### *6.8.7 Materials*

| Reagent | Consumables |
|---|---|
| Test devices Buffer | Disposable gloves<br>Laboratory coat<br>70% alcohol<br>Mask |

### *6.8.8 Storage And Stability*

Store Covid 19 rapid kit devoice 2-30$^{O}$C Protected from sunlight and should not be frozen

### *6.8.9 Safety*

i. Adhere to safety precautions as stated in the facility Safety manual
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections. **iv.** Refer to National infection prevention and control Guidelines for health waste **management and safety practice.**

### *6.8.10 Calibration*

Perform equipment calibration as per schedule

### *6.8.11 Quality Control*

Run known Negative and Positive in-house controls once a week.

The test strips contain a control line, which turns coloured if the run is valid.

### *6.8.12 Procedural Steps*

i. Peel off the aluminium foil seal from the sample processing tube containing the extraction buffer.
ii. After the sample collection, plunge the swab up and down in the sample processing tube for at least 15 seconds, taking care not to spill the contents out of the tube.
iii. Remove the swab while pinching wall of the tube with the swab and rotating the swab, to extract the liquid from the swab. iv. Firmly attach the dropper lid to the top of the sample processing tube.
v. Remove the test cassette from the sealed pouch. vi. Sample adding: Reverse the sample processing tube, holding the tube upright, and slowly add 3-4 drops to the sample ole (S) of the test cassette then start the timer.
vii. Timing observation: judge the result 15 minutes after sample adding; do not observe the results after 30 minutes later.
viii. After the test, put the medical wastes into the biosafety bag.

### *6.8.13 Biological Reference Interval*

Not Applicable

### *6.8.14 Interpretation and Reporting of Results*

**Results interpretation**

**A Positive:** Two distinct coloured bands appear on the strip.

**Negative:** Only one distinct coloured band on the strip.

**Invalid:** If no control band is seen.

**Reporting of results**

Report negative results as SARS-COV-2 – Negative.

Report positive result as SARS-COV-2 – Positive

**Critical value**

Any positive results

### *6.8.15 Limitation of the Procedure and Sources of Error*

i. The kit is not intended for testing liquid sample such as wash or aspirate sample or swab in transport media as a result can be compromised by over dilution.
ii. Insufficient sample volume or incorrect procedural techniques are the most likely reason for control line failure

### *6.8.16 Performance Characteristics*

Refer manufacturer reagent kit insert for sensitivity and specificity

### *6.8.17 Supporting DocumentsS*

Sample collection manual

### *6.8.18 References*

Manufacture package insert kit for SARS COV-2

## 6.9 PROCEDURE FOR DENGUE VIRUS ANTIBODY DETECTION RAPID TEST

### *6.9.1 Purpose*

This procedure provides details instruction for screening of dengue IgG, IgM antibody by using rapid test strip as an aid in the diagnosis of infection with Dengue virus

### *6.9.2 Scope*

This procedure is used in serology section when performing rapid *Dengue* antibody rapid tests.

### *6.9.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.9.4 Principle*

Dengue test utilizes immunochromatography whereby mouse anti- human IgM and human IgG antibodies are immobilized on the nitrocellulose membrane respectively, as two individual test lines (IgM line and IgG line) in the test window of the test device. The IgG line in the test window is closer to the sample well and followed by IgM line. As the test sample flows through the membrane within the test device, the coloured-dengue specific recombinant antigen-colloidal gold conjugate complexes with specific antibodies (IgM and or IgG) of dengue virus if present in the sample. This complex moves further on the membrane to the test region where it is captured by the anti-human IgM and or human IgG antibodies coated on the membrane leading to formation of a coated band, which indicates a positive test results. Absence of the coloured band in the test window indicates a negative test result. A built in control line will always appear in the test window when the test has performed properly regardless of the presence or absence of anti-Dengue virus antibodies in the sample. Dengue NSI antigen test is a solid phase immunochromatographic assay. As the test sample flows through the membrane within the test device and mobilize the gold anti-NSI conjugate that it is coated on the conjugate pad if NSI it is present then the result it is the formation of coloured band of the test (T)line region

### *6.9.5 Sample Requirements*

Plasma/whole blood, Serum (2-5ml)

**NOTE 1:** To obtain serum, Centrifuge blood collected in plain red top tube at 3000rpm/RCF per 3 minutes

**NOTE 2:** To obtain Plasma, Centrifuge blood collected in EDTA tube at 3000rpm/RCF per 3 minutes

### *6.9.6 Equipment*

Timer ,Centrifuge ,refrigerator and Thermometer

### *6.9.7 Materials*

| **Reagents** | **consumables** |
|---|---|
| • Dengue IgG/IgM antibody and NSI antigen Cassette<br>• Buffer<br>• Transfer pipette for dengue NSI<br>• Capillary pipette for Dengue IgG/IgM<br>• Known Positive control,<br>• Known Negative control | • Marker pen<br>• Examination Gloves<br>• Gauze |

### *6.9.8 Storage and Stability*

i. The test kit should be stored at 15-25ºC in the sealed pouch for the duration of the shelf-life (refer to manufacturer instruction)
ii. If the samples are not to be tested they should be refrigerated immediately at 4 - 8 ºC
iii. If storage periods > 5 days the sample should be frozen at -20$^0$C

### *6.9.9 Safety*

i. Adhere to safety precautions as stated in the Safety manual/IPC guideline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *6.9.10 Calibration*

Perform calibration of equipment (Timer ,Centrifuge ,refrigerator and Thermometer)as per calibration schedule

### *6.9.11 Quality Control*

i. Control samples weather commercial or in-house made are run the same way as patient sample on weekly bases and whenever a new kit is opened.
ii. Serology Section head should review Quality control records

### *6.9.12 Procedural Steps*

i. Bring the samples and the test components to room temperature if refrigerated or frozen. Mix the sample well prior to assay
ii. Remove the test strip from the foil pouch and use it as soon as possible
iii. Use transfer pipette to transfer sample by depressing the bulb of the pipette
iv. If capillary used withdraw 5ul of sample, the black bar near the opening end of pipette indicates the required 5ul of sample.
v. Drop the sample in the corner pointed by S1»
vi. Hold the pipette in a vertical position over the left “S” sample well on the device
vii. Transfer 2 drops of sample into well
viii. Dispense 2 drops of sample buffer to the right ”S” sample well
ix. Read result at the end of 20 minutes

### *6.9.13 Biological Reference Interval*

Not Applicable

### 6.9.14 Interpretation and Reporting of Results

**Interpretation** of results

**NEGATIVE**

- If only the “C” line is developed, the test indicates that no detectable antibodies to dengue are present in the specimen

**POSITIVE**

- Lines showed to control and NSI –NS1 POSITIVE during window period
- Lines showed to control and IGM-IgM Positive Chronic dengue
- Lines Showed to control and IgG- IgG Positive during early infections BUT has been treated

**INVALID**

If NO line is developed at “C”, the assay is invalid regardless of colour development on the “T” line. Repeat the test **Reporting of results**

Report results as: *Dengue* - Negative or *Dengue* – Positive

***Critical value***

Positive findings

### *6.9.15 Limitation of the Procedure and Sources of Error*

This kit is intended ONLY for testing of individual samples. Don’t use it for testing of cadaver samples, saliva, urine or other blood samples or pooled (mixed) blood

### *6.9.16 Performance Characteristics*

Refer manufacture kit insert for specificity and sensitivity. Also Method verification of this procedure should be done and that the report should be referred to verify compliance to this requirement. for Dengue rapid test procedure

#### *6.9.17 Supporting Documents*

Sample collection manual

#### *6.9.18* References

Dengue IgG/IgM antibody + NSI antigen Cassette Test Rapid Test Strip Package insert.

### 6.10 PROCEDURE FOR PLAGUE RAPID TEST

#### *6.10.1 Purpose*

This procedure provides details instruction of detecting acute bacterial infection caused by Yersinia pestis

#### *6.10.2 Scope*

This procedure is used in serology section when performing rapid *Plague* rapid tests(F1RDT)

#### *6.10.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

#### *6.10.4 Principle*

F1RDT detect pathogen-specific antigens (the F1 capsular antigen, which is part of the outer surface of *Yersinia pestis*, the bacteria causing plague) in a small quantity of different body fluids through lateral flow immunochromatography. The test is simple to perform and provides a result within 15 minutes. It can be performed in the pus contained in the buboes (swellings), or in the sputum (mucous coughed up from the respiratory tract) of people with suspected pneumonic plague.

#### *6.10.5 Sample Requirements*

Bubo aspirate, urine, and sputum,serum

#### *6.10.6 Equipment*

Centrifuge, timer, thermometer

### *6.10.7 Materials*

| Reagents | Consumables |
|---|---|
| F1RDT test kit | • Marker pen<br>• Examination Gloves<br>• Gauze |

### *6.10.8 Storage and Stability*

Refer to manufacturer storage instructions of test devices and samples

### *6.10.9 Safety*

i. Adhere to safety precautions as stated in the Safety manual/IPC guideline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *6.10.10 Calibration*

Perform calibration of equipment (Timer ,Centrifuge and Thermometer)as per calibration schedule

### *6.10.11 Quality Control*

Refer to manufacturer insert package

### *6.10.12 Procedural Steps*

Refer to manufacturer insert package

### *6.10.13 Biological Reference Interval*

Not Applicable

### *6.10.14 Interpretation and Reporting of Results*

**Interpretation** of results

Refer to manufacturer insert package **Reporting of results**

Report results as: *Plague* - Negative or Plague – Positive Critical value

Positive findings

### *6.10.15 Limitation of the Procedure and Sources of Error*

F1RTD test needs to be combined with other laboratory evaluations to confirm the diagnosis

### *6.10.16 Performance Characteristics*

Method verification of this procedure should be done and that the report should be referred to verify compliance to this requirement. of this procedure

### *6.10.17 Supporting Documents*

Sample collection manual

### *6.10.18 References*

Chanteau S, Rahalison L, Ratsitorahina M, Mahafaly, Rasolomaharo M, Boisier P, et al. Early diagnosis of bubonic plague using F1 antigen capture ELISA assay and rapid immunogold dipstick. *International Journal of Medical Microbiology* 2000;290(3):279-83.

## 6.11 PROCEDURE FOR HELICOBACTER PYLORI ANTIGEN TEST

### *6.11.1 Purpose*

This procedure provides instructions for the rapid detection of *Helicobacter pylori* antigen in human stool sample.

### *6.11.2 Scope*

This procedure is used in serology section to all rapid *H. pylori* antigen tests.

### *6.11.3 Responsible*

Qualified, trained, and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.11.4 Principle*

This is a ready to use test that is based on the homogeneous membrane system technology with latex microspores to perform the test, an aliquot of diluent sample is added to sample well of the test cassette. The sample flows through a label pad containing Pylori antibody coupled to red –colored colloidal gold. In the presence of antigens, they bind to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. These complex moves on the nitrocellulose membrane by capillary action towards the test line region on which Pylori specific to the antibody on the membrane in the form of a line. A second red control line will always appear in the results windows to indicate that the test has been correctly performed and the test device functions properly. If pylori is not present or lower than the detection limit of the test, only the control line will be visible. If the control line does not develop, the test in invalid.

***6.11.5 Sample Requirements*** Stool sample.

***6.11.6 Equipment***

Timer

***6.11.7 Materials***

| **Reagents** | **consumables** |
|---|---|
| Test cassette dilution buffer<br>-Known Positive control,<br>-Known Negative control | Marker pen<br>Examination Gloves |

***6.11.8 Storage and Stability***

I. Store un opened test device at 2- 30℃.if stored at 2-8℃, ensure that the test device is brought to room temperature before opening. Do not freeze the kit or expose the kit over 30℃. (refer to manufacturer instruction)
II. Store sample at 2-8℃ for up to 72 hours.

***6.11.9 Safety*** iv. Adhere to safety precautions as stated in the Safety manual/IPC guideline

v. All personal protective equipment (PPE) must be worn when performing this procedure.
vi. All samples must be regarded as potentially infections.

***6.11.10 Calibration***

Perform calibration of equipment as per calibration schedule

***6.11.11 Quality Control***

Analyse known Negative and Positive in-house controls once every week and whenever a new kit is opened.

***6.11.12 Procedural Steps***

**i.** Remove the test device from its foil pouch by tearing along the notch and use as soon as possible
**ii.** Unscrew the cap of the sample collection tube. **iii.** Randomly stab the sample collection stick into the fecal sample at list 6 different sites

iv. Return the sample collection stick into the sample tube and tighten the cap
v. Shake the sample collection tube vigorously to mix the sample and the extraction buffer
vi. Hold the sample collection tube upright and break off the tip of the sample collection tube, invert the sample collection tube and transfer two drops of the mixture into the sample pad of the test strip then start timer
vii. Wait 10-15 minutes and read the results for the colored lines to appear

viii. Do not read result after 15 minutes

### *6.11.13 Biological Reference Interval*

Not Applicable

### *6.11.14 Interpretation and Reporting of Results*

**Interpretation** of results

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

Positive - In addition to a pink coloured control (C) band, a distinct pink coloured band will also appear in the tests(T) region

Invalid – a total absence of colour in both regions or no coloured line appears on the control (C) region is an indication of procedure error or the tests deterioration. Repeat the test with a new kit.

**Reporting of results**

Report results as: H pylori - Negative or H pylori – Positive

### *6.11.15 Limitation of the Procedure and Sources of Error*

1.1. The test is a qualitative assay and is not for quantitative determination of antibodies concentration levels in human stool only

1.2. The results obtained should only be interpreted in conjunction with other diagnostic results and clinical information.

**1.3.** A negative result can occur if the quantity of the. Pylori antigen presence in the sample below the detection limits of the assay, or the antigen that are detected are not present during the stage of diseases in which a sample is collection.

### *6.11.16 Performance Characteristics*

Refer manufacture kit insert for specificity and sensitivity. Also Method verification of this procedure should be done and that the report should be referred to verify compliance to this requirement. for H pylori procedure

### *6.11.17 Supporting Documents*

Sample collection manual

### *6.11.18 References*

Manufacturer Kit insert for H pylori

## 6.12 PROCEDURE FOR HELICOBACTER PYLORI ANTIBODY RAPID TEST

### *6.12.1 Purpose*

Rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG) anti-Helicobacter pylori (H. pylori) in human serum or plasma. It is used as an aid in the diagnosis of infection with *H. pylori*

### *6.12.2 Scope*

This procedure is used in serology section when performing rapid *H. pylori* antibody tests.

### *6.12.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for implementing this test procedure.

The Head serology is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.12.4 Principle*

The *H. Pylori* rapid Test device is a lateral flow chromatographic assay based on the Principle of the antibody – sandwich technique. The membrane is pre coated with *H.pylori* Antigen on the test line region of the test. During testing *H. pylori* Antibodies in the serum or plasma sample reacts with coated antigen and migrates upward on the membrane chromatography by capillary action to the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result

### *6.12.5 Sample Requirements*

Plasma/whole blood, Serum (2-5ml)

**NOTE 1:** To obtain serum, Centrifuge blood collected in plain red top tube at 3000rpm per 3 minutes

**NOTE 2:** To obtain Plasma, Centrifuge blood collected in EDTA tube at 3000rpm per 3 minutes

### *6.12.6 Equipment*

Timer, Centrifuge, refrigerator and Thermometer

### *6.12.7 Materials*

| **Reagents** | **consumables** |
|---|---|
| Test cassette/strips buffer<br>-Known Positive control,<br>-Known Negative control | Marker pen<br>Examination Gloves |

### *6.12.8 Storage and Stability*

III. Store unopened test device at 2- 30℃. If stored at 2-8℃, ensure that the test device is brought to room temperature before opening. Do not freeze the kit or expose the kit over 30℃. (refer to manufacturer instruction)

IV. Store sample at 2-8℃ for up to 72 hours.

***6.12.9 Safety*** vii. Adhere to safety precautions as stated in the Safety manual/IPC guideline

viii. All personal protective equipment (PPE) must be worn when performing this procedure.

ix. All samples must be regarded as potentially infections.

### *6.12.10 Calibration*

Perform calibration of equipment (Timer, Centrifuge, refrigerator and Thermometer)as per calibration schedule

### *6.12.11 Quality Control*

i. Analyse known Negative and Positive in-house controls the same way as sample testing procedure once every week and whenever a new kit is opened.

ii. Serology Section head should review Quality control records

### *6.12.12 Procedural Steps*

**i.** Remove the test device from its foil pouch by tearing along the notch and use as soon as possible

**ii.** Place the test strip on a clean and level surface, hold the dropper vertically and transfer 1 drop of plasma/serum to the sample pad.

**iii.** Add 1 drop of buffer, then start the timer

**iv.** Read the results in 10-15 minutes

### *6.12.13 Biological Reference Interval*

Not Applicable

### *6.12.14 Interpretation and Reporting of Results*

**14.1 Interpretation** of results

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

Positive - In addition to a pink coloured control (C) band, a distinct pink coloured band will also appear in the tests(T) region

Invalid – a total absence of colour in both regions or no coloured line appears on the control (C) region is an indication of procedure error or the tests deterioration.

Repeat the test with a new kit.

**14.2 Reporting of results**

Report results as: *H pylori* - Negative or *H pylori* – Positive

**14.3** Critical value Not applicable

### *6.12.15 Limitation of the Procedure and Sources of Error*

- The test is for in vitro diagnostic use only
- The test should not be used as the sole criteria for the diagnosis of pylori infection since it only indicates the presence of antibodies in the sample

### *6.12.16 Performance Characteristics*

Refer manufacture kit insert for specificity and sensitivity. Also Method verification of this procedure should be done and that the report should be referred to verify compliance to this requirement. for H. pylori procedure

### *6.12.17 Supporting Documents*

Sample collection manual

### *6.12.18 References*

Manufacturer Kit insert for H pylori

## 6.13 PROCEDURE FOR BRUCELLA ANTIBODY DETECTION

### *6.13.1 Purpose*

This procedure provides instructions for Qualitative detection of antibodies of all isotopes against Brucella species.

### *6.13.2 Scope*

The procedure is used in the serology section in the diagnosis of brucellosis.

### *6.13.3 Responsible*

Qualified, registered and competent health laboratory practitioners are responsible for implementing this test procedure. Section heads are responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.13.4 Principle*

This test is based on antigen /antibody reaction. The smooth, attenuated stained Eurocell antigen suspensions are mixed with the patient's serum. Specific 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.

### *6.13.5 Sample Requirement*

Serum sample is prefarable for this procedure. Allow blood to clot and Centrifuge the sample at 3000rpm for 5 minutes

### *6.13.6 Equipment*

Centrifuge, Pipettes, Shaker and Stop watch

### *6.13.7 Materials*

Stained Eurocell-A/ Eurocell -M Antigen suspensions, Slide Test, 70% alcohol, Known Positive control, Known Negative control, Marker pen, Examination Gloves.

### *6.13.8 Storage and Stability*

Reagent should be stored at 2-8°C. Sample can be stored at room temperature for 4hrs then can be stored at 2-8°C If serum separated can be stored at -20°C for 1year.

### *6.13.9 Safety*

i. Adhere to safety precautions as stated in the facility Safety manual
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections. iv. Refer to National infection prevention and control Guidelines for health care services in Tanzania

### *6.13.10 Calibration*

Calibration of Centrifuge should be done as per schedule.

**Maintenance**

Maintenance of Centrifuge should be done as planned.

### *6.13.11 Quality Control*

Run the positive and negative controls sample daily before performing patient samples or run QC Parallel with patient sample.

### *6.13.12 Procedural Steps*

**i.** Prior to the start bring all reagents to room temperature (20 to 25˚C). ii. Shake and mix the Eurocell antigen suspension well before dispensing.
iii. Place one drop of positive and negative control onto the reaction circle of glass slide.
iv. Place 80ul of saline onto the next reaction circle of the glass slide.
v. Place 80ul of patient serum to be tested onto the next reaction circle. vi. Add one drop of the appropriate Eurocell antigen suspensions in each of the above circles. vii. Mix contents of each circle uniformly over the entire circle with

separate mixing sticks. viii. Gently rock the slide back and forth/observe for agglutination macroscopically for one minute

### *6.13.13 Biological Reference Interval*

Not applicable

### *6.13.14 Interpretation and Reporting of Results*

**Interpretation of results**

Agglutination is a **POSITIVE** test results and indicates the presence of specific antibodies to Brucella in the patient's serum. No agglutination is a **NEGATIVE** test results

**Reporting of results**

Report results as Brucella antibody – NEGATIVE or Brucella antibody – POSITIVE

### *6.13.15 Limitation of the Procedure and Sources of Error*

The test cannot distinguish between past infection and current infection

### *6.13.16 Performance Characteristics*

Refer to the verification report of this procedure

### *6.13.17 Supporting Document*

Result management procedure

### *6.13.18 References*

Monica Cheesbrough: District Laboratory Practice in Tropical Countries, Vol 1, Tropical Health Technology, 1998.

URIT 560 Urine Analyzer– User Manual for Urine Chemistry Analyzer

Practical Laboratory Manual-Jane Carter and Orgenes Lema

## 6.14 PROCEDURE FOR SALMONELLA TYPHI ANTIBODIES QUANTIFICATION METHOD

### *6.14.1 Purpose*

This procedure provides instructions for in vitro detection and quantitative estimation of specific antibodies to salmonella present in Human serum

### *6.14.2 Scope*

The procedure is used in serology section when performing widal test by slide method.

### *6.14.3 Responsible*

Qualified full registered and competent laboratory practitioners are responsible for implementing this test procedure.

The Head of serology section is responsible for ensuring the effective implementation and maintenance of this procedure.

### *6.14.4 Principle*

This test is based on the Principle of direct agglutination reaction. The smooth suspension of killed salmonella bacilli carries homologous O and H antigens. When patient serum (containing antibodies to *S typhi* and *S paratyphi)* is incubated with respective antigens, visible agglutination occurs. Arising titre of antibodies is indicative of Enteric fever

### *6.14.5 Sample Requirements*

Serum sample is preferable for this procedure. Allow blood to clot

Centrifuge the sample at 3000rpm for 5 minutes

### *6.14.6 Equipment*

Timer, Centrifuge, Pipettes, Water bath

### *6.14.7 Materials*

Stained salmonella antigen set, Stained salmonella antigen S typhi “O”, Stained salmonella antigen S typhi “H”, Disposable gloves, pipettes, White Tile or slide, Sample rack, Test tube rack, 5%Sodium hypochlorite to wipe and disinfect the spills and Marker Pen

### *6.14.8 Storage and Stability*

Reagent should be stored at 2-8°C

Sample can be stored at room temperature for 4hrs then can be stored at 2-8°C if serum separated can be stored at -20°C for 1year.

### *6.14.9 Safety*

i. Decontaminate working surfaces twice daily, in the morning and afternoon

ii.

iii. Adhere to safety precautions as stated in the facility Safety manual

iv. All personal protective equipment (PPE) must be worn when performing this procedure.

v. All samples must be regarded as potentially infections. vi. Refer to National infection prevention and control Guidelines for health care services in Tanzania,

### *6.14.10 Calibration*

Centrifuge should be calibrated as per schedule.

#### 1.1. Maintenance

Maintenance of Centrifuge should be done as planed

### *6.14.11 Quality Control*

Analyze the positive and negative controls sample daily before performing patient samples or run QC Parallel with patient sample.

### *6.14.12 Procedural Steps*

**Rapid slide test (Widal screening test)**

i. Clean the glass slide or white tile provided in the kit and wipe
ii. Place 1 drop of undiluted serum to be tested in each of the first two circle (1-2)
iii. Add one drop of antigen “O” and “H” in circles 1,2 respectively
iv. Mix the contents of each circle with separate stick and spread to fill the entire circle area
v. Rock the slide for one minute and observe for agglutination
vi. If agglutination is visible within one minute then proceed for quantitative estimation **Quantitative slide/white tile test**

| **Circle** | **Serum Volume** | **Appropriate Antigen Drop** | | **Titre** |
|---|---|---|---|---|
| 01 | 0.08ml | 1 drop | Mix and Rotate for one minute and observed agglutination | 1:20 |
| 02 | 0.04ml | 1 drop | | 1:40 |
| 03 | 0.02ml | 1 drop | | 1:80 |
| 04 | 0.01ml | 1 drop | | 1:160 |
| 05 | 0.005ml | 1 drop | | 1:320 |

### *6.14.13 Biological Reference Interval*

- When value of O and H antigen are less than 1:160 NEGATIVE
- When value of O and H antigen are greater than 1:160 POSITIVE

### *6.14.14 Interpretation And Reporting Of Results*

i. Salmonella typhi O: 1:(Respective titre)
ii. Salmonella typhi H: 1 :(Respective titre)

**Rapid/ white tile widal test**

Granular agglutination in case of “O” and flocculating agglutination in case of “H” indicate positive reaction **Quantitative slide/white tile test**

A diagnostic titre of 1:80 suggest positive results

### *6.14.15 Limitation of the Procedure and Sources of Error*

Rapid slide tests or quantitative slide tests are non-specific type of test. The positive results should be further confirmed by tube test and other microbiological investigations

### *6.14.16 Performance Characteristics*

Refer data for verification report

### 6.14.17 Supporting Documents

Not applicable

### *6.14.18 References*

Reagent package insert for widal test

## 6.15 PROCEDURE FOR CHORELA RAPID DIAGNOSTIC TEST

### *6.15.1 Purpose*

This procedure provides instructions for performing rapid Bioline Cholera Ag O1/ 0139 test

### *6.15.2 Scope*

The procedure is used for performing rapid Bioline Cholera Ag O1/ 0139 test in Microbiology section.

### *6.15.3 Responsible*

Qualified, trained and competent health laboratory practitioners are responsible for performing this test

### *6.15.4 Principle*

Cholera antigen test contains a membrane strip which is precoated with mouse monoclonal anti- *vibrio cholera* O1 antibody on test line O1 region and with the device. These line in result window are not visible before applying any specimen. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple test line will be visible in the result window if *vibrio cholera* O1 and/or O139 antigens are present in the specimen.

When a specimen is added to the test, vibrio cholera O1 and O139 antigens in the specimen react with colloidal gold-labelled *V. Cholerae* O1 and *V. Cholerae* O139 specific antibodies and form a complex of antigen-antibody colloidal gold conjugates. As this complex migrates along the length of the result window by capillary action, the complex is captured by the mouse monoclonal *V. cholerae* 01 antibody on test line 01 (O1) and mouse monoclonal *V. cholerae* 0139 antibody in the test line 0139 (0139) across the result window and generates a colored line. In the absence of *V. cholerae* 01 and 0139 antigen in specimens, a complex is not formed and no colored test line appears in the result window of test device.

### *6.15.5 Sample Requirement*

Human stool sample

### *6.15.6 Equipment*

Refrigerator

### *6.15.7 Materials*

Disposable gloves, Timer, Biohazard bag, Test device, Sample collection tube with extraction buffer, Sample collection swab for solid stool samples and Sample collection dropper for liquid stool samples

### *6.15.8 Storage and Stability*

a) ***For best result stool sample should be tested as soon as possible after*** collection.
b) Extracted stool sample is stable for 72 hours when store in 2-8ºC
c) Do not use stool sample in transport media or preservatives

### *6.15.9 Safety*

a) Wear protective gloves while handling sample and wash hands after testing
b) Do not mix or interchange different specimens
c) Decontaminate and dispose all sample test kits and potentially contaminated materials in a biohazard container as if they were infectious.
d) Do not mix reagents of different lots or those of other products.

### *6.15.10 Calibration*

Not applicable

### *6.15.11 Quality Control*

a) the cholera antigen rapid test has three test lines O1, O139 and control line. The control line is used for procedural control and shows that the diluent has been applied successfully and that the active ingredients of main components on the strip are functional.
b) Use samples with known results (positive and negative) to test each test kit batch before use and ensure the results correlate with the respective control samples.

### *6.15.12 Procedural Steps*

**Specimen Collection**

**In case of solid stool sample.**

a) Loosen the filter cap of the specimen collection tube.
b) Collect the sufficient stool sample (about 50mg) from different 4-5 sites of stool specimens using the specimen collection swab.
c) Immediately place the swab into the specimen collection tube.
d) Vigorously mix the solution by rotating the swab at least 10 times against the side of the specimen collection tube.
e) Release as much liquid as possible from the swab by squeezing the sides of the swab as the swab is withdrawn.
f) Discard the swab and then assemble filter cap on the specimen collection tube.

**In case of liquid stool sample.**

a) Loosen the filter cap of the specimen collection tube.

b) Draw liquid specimens up to the fill line (about 300ul) using disposable dropper.
c) Immediately transfer liquid specimen into the specimen collection tube.
d) Discard the dropper and then assemble filter cap on the specimen collection tube.

### *6.15.13 Testing Procedure.*

a) Bring the test device and sample to reach a temperature between 15-30ºC for at least 30 minutes in case they were refrigerated.
b) Remove the test device form the foil pouch and place it on a flat, dry surface.
c) Shake the collection tube thoroughly to ensure proper mixing of the sample with extraction buffer.
d) Loosen the nozzle cap of the specimen collection tube.
e) Hold the collection tube vertically and dispense 3 drops(70ul) into the specimen well of test device.
f) Wait a minimum of 15 minutes then read results. Do not read test results after 15 minutes; reading results after 15 minutes can yield false results.

### *6.15.14 Biological Reference Intervals*

Not applicable

### *6.15.15 Interpretation And Reporting Of Results*

A coloured control line will appear in the left section of the result window to show that the test is working properly.

Coloured lines will appear in the middle and right section of the result window. These lines are test line 0139 and test line 01 (0139, 01).

**Negative Result**: The presence of only control line (C) within the result window indicates a negative result.

**Positive Result**: The presence of two lines as control line (C) and test line 01 (O1) within the result window indicates a positive result for *V. cholerae* 01 antigen. The presence of two lines as control line (C) and test line 0139 (0139) within the result window indicates a positive result for *V. cholerae* 0139 antigen.

The presence of three lines as control line (C) test line 01 (O1) and test line 0139 (0139) within the result window indicates a positive result for *V. cholerae* 01 and 0139 antigen.

**Caution**: The presence of any line, no matter how faint, the result is considered positive.

**Invalid Result**: If the control line (C) is not visible within the result window after performing the test, the result is considered invalid.

### *6.15.16 Limitations Of The Procedure And Sources Of Error*

a) A negative result does not exclude the possibility of *V. cholerae* 01 and/or 0139 infection in a patient. Failure to detect *V. cholerae* 01 and/or 0139 may be a result of factors such as collection of specimens at an improper time in the disease when few bacteria are present and improper sampling or handling of the specimen.
b) A positive result does not preclude the presence of other enteric pathogens. While the relationship between cholera and gastroenteritis is well established, concurrent infection with other microbial pathogens is possible. Additional microbiological tests should be performed in parallel with Bioline Cholera Ag O1/ 0139 test kit in order to exclude other possible causes of the illness.
c) Test results should be interpreted in conjunction with information available from epidemiological studies, clinical symptoms of the patient and other diagnostic procedures.

### *6.15.17 Performance Characteristics*

Refer to manufacture user manual

### *6.15.18 Supporting Documents*

Sample collection manual

### *6.15.19 References*

Refer to manufacture user manual

# CHAPTER 7: BACTERIOLOGY AND MYCOLOGY

## 7.1 POTASSIUM HYDROXIDE WET MOUNT PREPARATION

### *7.1.1 Purpose*

This procedure provides instructions for examination of wet preparations that is mainly used to examine samples and cultures for motile bacteria, C.S.F for capsulated yeast cells and for fungi.

### *7.1.2 Scope*

This procedure applies to the microbiology section and health laboratory practitioners in the laboratory settings.

### *7.1.3 Responsible*

Competent Health Laboratory Practitionersare responsible for implementing this test procedure.

The Head of Microbiology/who asned is responsible for ensuring the effective implementation and maintenance of this procedure.

### *7.1.4 Principle*

A KOH preparation is used for Samples such as skin scrapings, nail, infected hairs, or for other Samples such as sputum to clear out background debris that may be confused with fungal elements. KOH dissolves proteinaceous tissues, including keratin, and renders them transparent. This enables fungi to be visualized more easily. The use of KOH is not necessary for Samples such as CSF where background debris is minimal.

### *7.1.5 Sample Requirements*

KOH preparation is used ideally in suspected cases of dermatophytosis, i.e., fungal infection of skin, hair, or nails. Also used for specimen such as sputum, pus, and urine sediment.

***7.1.6 Equipment*** Microscope

### *7.1.7 Materials*

Potassium hydroxide 10 or 20%, Potassium hydroxide 10 g, Glycerol 10ml, Deionized water 80ml, (optional) helps prevent the KOH mount from drying. Dispense working solution in a dropper bottle. Microscope slides, 24 x 50 mm cover slips, sterile forceps, mounting needle

### *7.1.8 Storage and Stability*

Potassium hydroxide, 10 or 20% solution is stable for one year at room temperature

### *7.1.9 Safety*

Observe standard safety precautions when handling Samples. Refer to Safety Manual

Discard all used materials (e.g., sticks, pipettes, disposable forceps) in a bucket containing bleach.

***7.1.10 Calibration*** Not applicable

### *7.1.11 Quality Control*

Fungal spores may contaminate the KOH solution, and may give false positive results. Solution must be examined for signs of contamination (e.g., turbidity) before each use.

### *7.1.12 Procedure Steps*

i. Place the material to be examined on a glass slide, add a drop of 10% KOH
ii. Place a cover slip over the preparation. iii. Let stand for five to ten minutes. iv. If clearing is not complete, an additional ten minutes is necessary.

***Note: Keratinous (skin, nails) Samples should be left at room temperature for 20 to 30 minutes to allow digestion and "clearing" of the keratin, after clearing, press coverslip gently to make a thin mount.***

v. Examine the slide microscopically on low power and confirm observations with high power. Observe for hyphae, conidia, budding yeasts, spherules or sclerotic bodies, etc. Consult photomicrographs in appropriate references when necessary, to identify fungal structure.

***7.1.13 Biological Reference Intervals*** Not applicalbe

### *7.1.14 Interpretation and Reporting of Results*

**Interpretation**

Dermatophytes in skin or nail are seen as branching hyphae or arthrospores and often appear slightly greenish in color, with hyphae running across the colorless host cells. Most hyphae will be parallel-sided and around 2 µm in width. Yeasts are present as budding cells, pseudohyphae, or yeast mycelium.

In infections caused by dematiaceous fungi, the hyphae are often brown (dematiaceous septate hyphae).

Artifacts, such as fibers, may be distinguished from hyphae from the lack of septa, tapering ends, and size differences.

**Reporting of Results**

Report the type of fungal structure seen. Do not report quantity.

**Examples:** "Fungal elements seen (septate hyphae)"

"Fungal elements seen (yeast cells and pseudohyphae)"

Report negative results as: "No fungal elements seen"

Report as: "Fungal elements seen (*Malassezia* spp.)"

***7.1.15 Limitations of the Procedure and Sources of Errors***

i. Cotton swabs should not be used in preparing these slides as the cotton strands may resemble hyphae. ii. The contrast between unstained fungal elements that may be present and the background mounting fluid can be accentuated by narrowing the iris diaphragm to reduce the amount of incident light; or use of phase-contrast microscopy that can greatly enhance visualization of organisms. iii. KOH preparations are presumptive and should not be substituted for culture and further identification.

iv. KOH should not be stored in a glass bottle as it will leach minerals from the glass. The minerals will result in a cloudy, flocculant solution. This can be avoided by storing the KOH in plastic, polystyrene or other non-glass container.

v. Gentle heating may speed the activity of the KOH, but it may be harmful to the specimen if overdone. vi. KOH preparations are not permanent; the reagent will eventually destroy the fungi. The addition of small amount of glycerol to the preparation will preserve it for several days.

vii. KOH combined with calcofluor white is a more sensitive method, but a fluorescent microscope with appropriate filter is required.

viii. India ink, added to the KOH, stains fungal elements and helps them stand out against the background. Reagent is prepared as follows: Make a 10% KOH solution in Parker Super Quink Ink, permanent blue black (50 ml of ink + 5 g of KOH pellets). Centrifuge KOH-ink solution at 2,000 x g for ten minutes. Pour supernatant into plastic (not glass) sterile tube. Store at room temperature.

***7.1.16 Performance Characteristics*** Not applicable

***7.1.17 Supporting Documents*** Not applicable

***7.1.18 References***

Lynne S. Garcia, Henry D. Isenberg. Clinical Microbiology Procedures Handbook. 3rd edition.
American Society for Microbiology. Washington DC, USA. 2010.

Guidelines on Standard Operating Procedures for Laboratory Diagnosis of HIV/AIDS Opportunistic Infections in Patients. WHO Regional Office for Southeast Asia. New Delhi. June 2001
Bailey and Scott's Diagnostic Microbiology. 13th edition. Mosby, Inc. St. Louis, Missouri, USA. 2013.

## 7.2 PROCEDURE FOR ZIEHL NIELSEN STAIN

### *7.2.1 Purpose*

This procedure provides instructions for performing Ziehl-Nielsen stain of sputum or other body fluid samples.

### *7.2.2 Scope*

This procedure is to be used for detection of Acid Fast Bacilli in sputum or other body fluid samples in the Laboratory

### *7.2.3 Responsible*

The section heads and technical staffs are responsible for implementing this procedure.

### *7.2.4 Principle*

This procedure is used to stain mycobacterium tuberculosis and mycobacterium leprae. These bacteria are also called acid fast bacilli. They stain with carbolfuschin, which is a red dye. They retain the dye when treated with acid, which is because of the presence of mycolic acid in their cell wall.

### *7.2.5 Sample Requirements*

The Sputum and body fluids such as CSF, synovial, pericardial, synovial, ascitic, blood, pus, bone marrow, tissue biopsies or pleural fluid samples.

### *7.2.6 Equipment*

Centrifuge (when necessary), Bunsen burner/spirit lamp, Light Microscope and Biosafety cabinet

### *7.2.7 Materials*

ZN stains reagents: 1% Carbol Fuchsin stain, 20% Sulphuric acid and 0.125% Methylene blue. Acid Fast Bacilli Positive and negative Control slides for zn.

Personal protective gears, Gauze, Glass slides, Staining racks, Drying rack, Clean Glass slides, Applicator stick, Micropipette or Pasture pipettes,sputum container,timer

### *7.2.8 Storage and Stability*

Sputum samples should be proccesed within 3days if stored at room temperature and if not possible store it at 2 to 8°C for 10 days.

### *7.2.9 Safety*

Decontaminate working surfaces as recommended by IPC Guidelines.

All personal protective equipment (PPE) must be worn when performing this procedure.

All samples must be regarded as potentially infections.

Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### *7.2.10 Calibration*

Centrifuge and safety cabinety should be calibrated as per schedule

### *7.2.11 Quality Control*

Perform IQuality control by Smearing and staining known Negative and positive sample /EQA before examining any patient sample every day. Perform lot to lot for every new batch of reagent received

### *7.2.12 Procedure Steps*

i. Place the fixed slides with smear upwards on a staining rack over a sink about 1 cm apart.
ii. Flood the smear with filtered carbol fuchsin staining solution.
iii. Prepare a torch by dipping its cotton wool end in burning spirit and light it. Use only a few drops on acid alcohol or 70% v/v ethanol or methanol.
iv. Heat the slide keeping the torch a little below the slide and moving it continuously forth and back along the line until steam arises, repeat twice at intervals of 3-5 minutes. Do not overheat. Allow slides to stand for at least 30 minutes.
v. Tilt the slide using forceps to drain off the staining solution.
vi. Rinse the slide well with clean water from a beaker (or running tap water).
vii. Pour the 20% Sulphuric acid or 3%Hcl over the smears, covering them completely. Allow to act for 3 minutes.
viii. Tilt the slide with forceps to drain off the acid solution; then gently rinse the slide again with clean water.
ix. Repeat covering by sulphuric acid or Hcl solution and rinsing once for smears that are still red.
x. Flood smear with methylene blue solution for 1 minute.
xi. Tilt the slide with forceps draining off the Methylene blue solution. xii. Wash with clean water.

xiii. Using forceps take the slide from rack, drain off water and stand the slide on the edge to air dry on the drying rack.

xiv. Examine the smear microscopically first with the 40x objective to see the distribution of material, then with the oil immersion objective to look for Acid Fast Bacilli . Open fully the condenser iris when using the oil immersion lens. After examining a positive smear, the oil immersion objective must be wiped clean.

***Note: The stained smear should show a light blue colour from methylene blue. If the smear is dark blue, it usually indicates that the smear is too thick.***

### *7.2.13 Biological Reference interval*

Not applicable

### *7.2.14 Interpretation and Reporting of Results*

Acid Fast Bacilli appears as Red, straight or slightly curved rods, occurring singly or in small.

If any definite red bacilli are seen, report the smear as Negative If no red bacilli are seen in at least 100 fields and 'Acid Fast Bacilli positive' if give an indication of the number of bacteria present as follows:

| | |
|---|---|
| More than 10 AFB/HPF field | Report + + + OR +3 |
| 1 – 10 AFB/HPF field | Report + + OR +2 |
| 10 - 99 AFB/100 fields | Report + OR +1 |
| -9 AFB/100 fields | Report the exact number/100 |
| If no red bacilli are seen in at least 100 fields | Report as negative |

### *7.2.15 Limitation of the Procedure and Sources of Error*

i. Re use of containers or positive slides.
ii. Contaminated stain prepared with water containing environmental mycobacteria.
iii. Use of scratched slides.
iv. Acid Fast Bacilli floated off one slide and became attached to another during staining procedure because of no distance between each slide.
v. Poor quality of staining solutions.
vi. Taking improper portion of sample for smear preparation. vii. Improper focal distance for examination.
viii. Use of poorly prepared staining solution. ix. Overheating during fixing.
x. Too long interval between staining and reading, especially when slides not kept in dark or poorly stained.

***7.2.16 Performance Characteristics*** Not applicable

### *7.2.17 Supporting Document*

Patients Results Register - TB 05

Internal Quality Control review form

### *7.2.18 References*

Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C., USA, 2nd edition, 2007.

Monica cheesbrough (2005). District Laboratory Practice in Tropical countries. Cambridge University Press, New York, USA, 2nd edition, 2005.

WHO, (2003). Mannual of basic techniques for a health laboratory. Geneva. 2nd edition, 2003.

Manual of Clinical Microbiology. American Society for Microbiology (ASM), Washington D.C., USA. 9th edition, 2007.

## 7.3 PROCEDURE FOR AURAMINE O PHENOL STAINING

### *7.3.1 Purpose*

The Auramine O staining technique applies to identification of Acid Fast Bacilli in patient sample using fluorescence Microscopy which increase the rate of detection compared to the light microscopy.

### *7.3.2 Scope*

This procedure is to be used for detection of Acid Fast Bacilli in sputum or other body fluid samples in the Laboratory by using Auramine O reagents.

### *7.3.3 Responsible*

Competent Health Laboratory Practitioners are responsible for implementing this test procedure.

The Head Microbiology is responsible for ensuring the effective implementation and maintenance of this procedure

### *7.3.4 Principle*

The fluorochrome dye, Auramine-Rhodamine, forms a complex with mycolic acids contained in the acid fast cell wall of organisms which resist decolorization by acidalcohol. The counterstain, Methylene blue 0.3%, renders tissue and its debris non fluorescent, thus reducing the possibility of artifacts. The cells visualized under ultraviolet light appear bright yellow or reddish orange.

### *7.3.5 Sample Requirements*

Mucoid, purulent or blood stained samples.

### *7.3.6 Equipment*

Biosafety cabinet, Fluorescent microscopes, Timer, Bunsen burner/ Spirit lamp

### *7.3.7 Materials*

Auramine –O phenol 0.1%, Hydrochloric acid in Alcohol 0.5%, Methylene blue 0.3%, Microscopic slides, Slide holding box, Gloves, Gauze/Cotton wool, Beaker, Forceps Applicator sticks/Disposable, Pencil, Dry rack, Slide racks, wide mouth sputum container for routine samples or Falcon tube 50ml for referral samples.

### *7.3.8 Storage and Stability*

Sputum samples should be proccesed within 3 days if stored at room temperature and if not possible store it at 2 to 8°C for 10 days.

### *7.3.9 Safety*

Decontaminate working surfaces as recommended by IPC Guidelines.

All personal protective equipment (PPE) must be worn when performing this procedure.

All samples must be regarded as potentially infections.

Refer to National infection prevention and control Guidelines for health waste management and safety practice.

### *7.3.10 Calibration*

Centrifuge and safety cabinety should be calibrated as per schedule

### *7.3.11 Quality Control*

Internal Quality Control should be done by smearing and staining known Negative and positive sample before examining any patient sample every day. Perform lot to lot for every new batch of reagent received/prepared.

### *7.3.12 Procedure*

***Film Preparation***

i. Label the slides properly using a unique laboratory number
ii. Place the labeled slides, the samples and the applicator stick /Pasteur pipette in the Biological safety cabinet
iii. Match each slide with the corresponding sputum or sample container. iv. Proceed to smearing, taking the labeled slides and opening containers one by one, do the smearing from the center of the slide, outwards making small coil – like movements (A thin smear ,allow to air dry).
v. Select a small portion of purulent or muco-purulent material with the applicator stick for a direct sputum smear and then transfer it to the slide, if a stick is used, break it in two pieces and used ragged ends for dissecting sputum and for smearing.

vi. Spread the material carefully over the area equal to about 2×1 cm using repeated coil like movements, without touching the margins of the slides and should be in the middle.
vii. Make the smear as even as possible by continuing this process until no thick parts remain. Remove excess material with the second stick and discard in the biohazard bag.
viii. The thickness of the smear should be such that a newspaper can bared bye read through the dried smear held about 10cm above it (Translucent).
ix. Warm the slides on the slide warmer in the biological safety cabinet to dry and fix the smear for at least 30minutes.
x. Re-fix the smears by passing a flame under the slides before staining.
xi. If the slide warmer is not functioning, air dry the smear then fix them by passing a flame under the smear ,the smear should face upward ,do not over heat ,or else Acid Fast Bacilli staining will be poor.

**Fluorescent Microscopy Staining Procedure**

Place the slides on staining rack. IT IS A MUST to keep distance of at least 1cm between every slide. Otherwise there is a possibility that acid fast bacilli may cross contaminate the negative smears due to over flooding or splashes from positive smear to a negative smear

i. Cover the smear completely with filtered 0.1% auramine solution **Do not heat**
ii. Leave for 15 minutes iii. Wash the slides well with distilled water or running water iv. Pour the acid alcohol solution over the slides.
v. Allow to act for 2- 3 minutes.
vi. Gently rinse each slide with distilled or running water. vii. Repeat decolourization if macroscopically visible stains are still visible.
viii. Flood smear with 0.5% methylene blue counter solution for 1 minute .Time is critical because counterstaining for longer time may quench the acid fast bacilli
ix. Gently wash off counterstain with distilled or running water.
x. Stand the slides on edge to drain and air dry on the slide rack away from direct sun light.

**Examination**

i. Keep stained smears in the dark (box or folder) till reading, and read as soon as possible (within 24hours) since fluorescence fades quickly out of the box.
ii. Use 20 x objectives for scanning and 40x confirmation; scan the stained smear systematically from one side to another side
iii. One length has to be scanned before reporting a Negative. iv. Acid-fast bacilli appear bright yellow against the dark background material.
v. Store the slides in a slide box according to the study, following the laboratory Number as they will be needed for EQA.

***Note: Acid fast bacilli appear bright yellow against dark background, report as possible for Acid Fast Bacilli if at least one acid-fast bacillus was seen in a well stained smear, even if you think they might be other mycobacterium other than tubercle bacilli. Tubercle bacilli are quite variable in shapes from very short fragments to elongated types. They may be uniformly stained or with one or many gaps, or even granular. They occur singly or in small groups (coded), and rarely in large clumps. The typical appearance of bacillli are usually rather long and slender, straight or slightly curved rods.***

### *7.3.13 Biological Reference interval* Not applicable

### *7.3.14 Interpretation and Reporting of Results*

If fluorescent Acid Fast Bacilli are seen, report the smear as Acid Fast Bacilli positive, and give an indication of the number of bacilli present in plus signs (+ to +++).

The results have to be reported on the working sheet and on patients result register (MTB Register 05 if available).

If no fluorescent rods are seen, report the smear as (NO AFB SEEN).

| **Report** | **Fluorescence (200magnigication, one length 20x=field 200=HPF** | **Fluorescence (400magnigication, one length 40x =field 200=HPF** |
|---|---|---|
| Negative | Zero AFB/1 length | Zero AFB/1 length |
| Scanty | 1-29 AFB/1 Length | 1-19 AFB/1 Length |
| 1+ | 30-299 AFB/1 Length | 20-199 AFB/1 Length on average |
| 2+ | 10-100 AFB/ 1field on average | 5-50 AFB/1 field on average |
| 3+ | >100 AFB/ Field on average | >50 AFB/ Field on average |

**Critical values**

Presence of Acid Fast Bacilli

### *7.3.15 Limitation of the Procedure and Sources of Error*

i. Direct reagents to Sun light
ii. Poor reagent quality
iii. Using wrong reagent Concentration
iv. Using unfiltered reagent
v. Using expired reagent
vi. Poor sample quality

### *7.3.16 Perfomance characteristics* Not applicable

### *7.3.17 Supporting documents*

Patients Results Register - TB 05 Internal Quality Control

### *7.3.18 References*

International union against tubercle and lung diseases. The public health service national tuberculosis Reference laboratory and the national laboratory Network ,Paris 1998
Smithwick R.W laboratory Manual for acid fast microscopy .US Department of Health ,Education and Welfare,CDC,1979
Angra P, Becx-Bleumink M,Glipin C,et al,Ziehl Neelsen staining ,strong red on week blue, or weak red under strong blue Int J Tuberic Lung Dis 2007:11:1160-1

## 7.4 PROCEDURE FOR GRAMS STAINING

### *7.4.1 Purpose*

This procedure provides instructions on the steps to be followed when performing Gram staining

### *7.4.2 Scope*

This procedure will be used by all laboratory personnel perform gram staining to identify bacteria

### *7.4.3 Responsible*

It is the responsibility of the Head of Microbiology Section to ensure effectively implemented and maintained .

### *7.4.4 Principle*

Gram stain based on the ability of bacteria cell wall. When the bacteria are stained with primary stain (crystal violet) and fixed by the mordant (Iodine), Gram Positive bacteria retain the primary stain when decolorized by ethanol (alcohol) because the cell walls of gram positive bacteria have THICK layer of protein-sugar components called peptidoglycan and low lipid contents. Upon decolourization Gram positive bacteria causes thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevent the stain from existing the cell, therefore ethanol cannot remove crystal Violet-Iodine complex that is bound to the thick layer which peptidoglycan and appears BLUE. While Gram Negative bacteria are decolorized by ethanol. For the gram negative bacteria cell wall takes up the crystal violet-iodine complex but due to the thin layer of peptidoglycan and thick outer layer with form of lipids, crystal violate-iodine complex gets washed off, when they are exposed to decolorizer dissolves the lipids in the cell walls which allow the crystal violet-iodine complex to lead out of the cells then when again stained with counterstain (safranin) they pick up the safranin and appears red in color.

### *7.4.5 Sample Requirement*

Fresh collected Pus, urine sediment, CSF, sputum, other body fluids

### *7.4.6 Equipment*

Microscope, Timer, Bunsen burner/hot plate

### *7.4.7 Materials*

| Reagents | Consumables |
|---|---|
| • Crystal violet as primary stain<br>• Lugol's iodine as mordant<br>• 10% Acid/Acetone as decolourizer<br>• Neutral red/safranin as counter stain | • Gloves<br>• Gauze<br>• Waste bins<br>• Grass slides<br>• Applicator stick |

### *7.4.8 Storage and Stability*

i. Samples are stable at 2-8$^0$C for 7 daysdays
ii. store reagentas instructed by manufacturer

### *7.4.9 Safety*

i. Adhere to safety precautions as stated in the Facility Safety manual/ IPC guideline
ii. All personal protective equipment (PPE) must be worn when performing this procedure.
iii. All samples must be regarded as potentially infections.

### *7.4.10 Calibration*

Perform Equipment calibration as scheduled.

### *7.4.11 Quality Control*

Quality control is done daily when receiving/preparing a new batch of reagents. A known sample ATCC for gram positive Staphylococcus aureus and for gram negative Escherichia coli bacteria respectively are used.

### *7.4.12 Procedural Steps*

***Smear preparation and staining***

i. Slide with one end frosted should be used for making smears so that a lead pencil can be used to label the slide clearly.
ii. Use a sterile applicator stick to add one drop of normal saline on the slide.
iii. Use a sterile applicator stick to transfer one pure colony on the slide and make a smear iv. Allow smear to air dry on a flat safe place.
v. Flood the fixed smear with crystal violet for 30 seconds
vi. Decant crystal violet and rinse gently slide with running tap water
vii. Flood the slide with Lugol's iodine for 30 seconds

viii. Rinse off iodine gently with flowing tap water ix. Decolorize by letting the reagent flow over the smear while the slide is held at an angle or tilt slide.
x. Adjust decolourisations time to thickness of smear
xi. Remove excess decolorizer with gentle flow of tap water
xii. Flood with neutral red for 30 seconds
xiii. Remove excess neutral red with gentle flow of tap water
xiv. Drain slide and air dry in an upright position

**Reading smears**
Place the slide on the microscope and ensure that the smear is facing upward

### *7.4.13 Biological Reference Intervals*

Not Applicable

### *7.4.14 Interpretation And Reporting Of Results* **Interpretation of results**

- If Bacteria pick the color of the counter stain (Neutral red/dilute Carbolfuschin/safranin) THEN report as Gram Negative (Cocci, Bacilli/Rod depending on morphological observed/identified cells)
- IF stained bacteria pick the color of the primary stain (crystal violet) THEN report as Gram Positive (Bacilli/Rods or cocci depending on observed identified cells) **Reporting of results**
- Results should be reported as Gram Positive Rods or Cocci according to the morphology of the bacteria or
- Gram NEGATIVE Rods or Bacilli according to the shape of bacteria

### *7.4.15 Limitations Of The Procedure And Source Of Error*

Avoid over staining, Avoid over decolourization Do not use expired reagent.

### *7.4.16 Performance Characteristics*

Refer to method verification report of this procedure

### *7.4.17 Supporting Document* Sample collection manual

### *7.4.18 References*

Districtlaboratory practice in tropical countries. Part 2: AuthorMonica Cherbourg 6th edition

# CHAPTER EIGHT: MOLECULAR BIOLOGY

## 8.1 DIAGNOSIS OF MTB/RIF TESTING USING A TRUENAT MACHINE

### *8.1.1 Purpose*

The purpose of SOP is to describe the stepwise procedure for the rapid detection of Mycobacteria tuberculosis (MTB) and detection of rifampicin resistance using Truenat MTB/RIF test for use with the Truenat Dx system is a semi-quantitative realtime PCR in-vitro diagnostic test for: The detection of MTB DNA in sputum samples, detection of rifampicin resistance-associated mutations of the rpoB gene. The purpose of SOP is to describe the stepwise procedure for the rapid detection of Mycobacteria *tuberculosis* (MTB) and detection of rifampicin resistance using Truenat. MTB/RIF test for use with the TrueNat Dx system is a semi-quantitative real-time PCR *in-vitro* diagnostic test for; detection of MTB DNA in sputum samples and detection of rifampicin resistance-associated mutations of the *rpoB* gene.

### *8.1.2 Scope*

This SOP describes the use of the Truenat™ MTB Plus assay, a chip-based RealTime Polymerase Chain Reaction (PCR) test, for the semi-quantitative, detection and diagnosis of Mycobacterium tuberculosis complex bacteria (MTBC) in human sputum samples

### *8.1.3 Responsible*

Qualified and competent health laboratory practitioners are responsible for implementing this test procedure.

The section head is responsible for ensuring the effective implementation and maintenance of this procedure.

### *8.1.4 Principle*

The TrueNat assays utilize chip-based real-time micro-polymerase chain reaction (PCR) for detection of TB and RIF-resistance from Deoxyribonucleic Acid (DNA) that is extracted from sputum sample within an hour.

Truenat™ MTB Plus works on the principle of Real-Time Polymerase Chain Reaction where the sputum sample is first liquefied and lysed thereafter the extracted DNA from the sample is then amplified by the Truelab Real-Time microPCR analyser. The purified DNA is then dispensed into the reaction well of the Truenat™ MTB Plus chip and the test is started. **Description of the apparatus**

Trueprep AUTO Sample Prep device

Truelab™ Real Time micro PCR Analyser

**Figure1. Truenat Dx System hardware components**

### *8.1.5 Sample Requirements*

Sputum sample type, collected as either Spot or morning sputum sample

### *8.1.6 Equipment*

True prep AUTO Sample Prep device, Truelab Real Time micro PCR Analyser and Refrigerator

### *8.1.7 Materials*

| Extraction of DNA | Amplification of purified DNA | Materials required but not provided | Reagents and Solutions |
|---|---|---|---|
| • Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device<br>• Trueprep AUTO MTB Sample Pre-treatment Pack<br>• Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit | • Truelab Duo Real Time Quantitative micro PCR Analyzer<br>• Truenat™ MTB Plus micro PCR chip<br>• Truelab™ micro PCR printer<br>• Truepet SPA fixed volume (6 µl) Precision micropipette<br>• • DNase and RNase-free pipette tips with filter barrier | • Truenat™ Positive Control Kit - Panel I<br>• Powder free disposable gloves<br>• Two waste disposal containers, with lids, containing bleach solutions<br>• Timer<br>• Two waste bags<br>• Micro tube Stand<br>• . Cartridge Holder<br>• Two cryovials racks | • Liquefaction buffer<br>• Lysis buffer<br>• Conc. bleach and 70% alcohol |

***Note: Connect a new reagent pack to the Trueprep Auto v2 device by inserting the Plug-in Connector into the slot provided.***

***8.1.8 Storage and Stability*** store at room temperature for 3 days

Store at 2°C -8°C

***8.1.9 Safety***

- Treat all biological samples, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological samples should be treated with universal precautions.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and reagents.
- Wash hands thoroughly after handling samples and test reagents. Follow safety procedures for working with chemicals and handling biological samples, (See safety manual)
- Dispose used cartridges according to infectious waste material disposal guidelines.

***8.1.10 Calibration***

Calibration is perfumed as per schedule

***8.1.11 Quality Control***

- Use Truenat Positive Control Kit- Panel containing Positive Control and Negative Control or use PBS as a negative control and a known positive culture sample.
- Run positive and negative controls at least one time per month or ➢ When opening a new o test kit lot.
  - If the temperature of the storage area falls outside of 2-30˚C.
  - New user prior to performing testing on the clinical sample.
  - Accept patient results if the positive controls give positive results while negative controls give negative results. Corrective action should be taken in case of QC failure either by repeating the control and/or informing the supervisor.
  - Whenever a new shipment of test kits is received.
- Records QC results in the TB register.

***8.1.12 Procedural Steps***

**A. Equipment start-up procedure** Press the “Power” button to switch on the Truenat device.

Press ‘start’ and ‘eject’ simultaneously to reset when prompted to change the Reagent Pack and reset

**B. Sample Processing procedure**

**Prepare Sample and Extract DNA**

i. Wear gloves for sample handling.
ii. Label Sputum sample with patient details or lab ID

iii. Add 2 drops of liquefaction buffer to the sputum sample
iv. Close the cap and swirl gently to mix
v. Incubate for 10 minutes at room temperature. If sample is not pipetteable after 10 minutes, incubate for another 5 minutes with swirling at 2-minute intervals
vi. Transfer 0.5 ml of liquefied sputum sample into the lysis buffer bottle using a 1 ml transfer pipette
vii. Add 2 drops of liquefaction buffer into the lysis buffer bottle, swirl gently to mix and incubate for 3-5 minutes
viii. Remove the cartridge from the pouch, label it and place it on the cartridge stand. Take out the elute collection tube (ECT) and label it appropriately. Keep it aside for later use. Keep the elute transfer pipette in the pouch for later use.
ix. Transfer the entire contents of the lysis buffer tube to the sample chamber (black cap) of the cartridge using 3 ml transfer pipette
x. Switch "on" the Trueprep® AUTO v2 device. Press "eject" button to open and gently pull out the cartridge holder
xi. Place the cartridge in the tray, and gently push to close the cartridge holder. Press "start."

The device will beep at the end of the DNA extraction process (20 minutes), and the cartridge holder will eject automatically.

xiii. Gently pull out the cartridge holder, remove the cartridge, and place it on the cartridge stand.
xiv. Carefully pierce the elute chamber with the provided elute transfer pipette, and transfer the entire elute into the ECT. Discard the transfer pipette and cartridge

**C. Running a PCR TB Test**

i. Switch "on" the Truelab microPCR analyser by pressing the red button in the back right corner for 2 seconds. LED will glow in Green. Wait for 30-50 seconds for "boot-up screen" to appear followed by "home screen."
ii. Select USER ID, enter password. Press "Sign in" to Log in
iii. Select test profile "MTB" or "MTB Plus". To confirm selection tap "PROCEED" and enter patient details (referred by, patient ID, gender, patient name & age)
iv. Select sample type (sputum).
v. Open a TRUENAT™ MTB Plus chip pouch *Pull out the orange desiccant pouch and confirm that it is orange in colour.
vi. Gently take out the chip without touching white well portion and place it on the chip tray by aligning it in the slot provided
vii. Press "START TEST" on the screen. Chip tray opens. "Please Load Sample" will appear. (Don't press "YES" until chip loading is complete.
viii. Open the master mix tube, discard the stopper and place the tube in the micro tube stand. *Check for white cake at the bottom of the micro tube.

ix. Attach the 6ul micro tip provided in the pouch to the single push pipette.

x. Transfer 6ul of the elute from ECT into the master mix tube

xi. Allow the master mix to stand for 30 SECONDS to get a clear solution. *Do not mix by tapping, shaking or reverse pipette. *Do not discard the pipette tip.

xii. Transfer the elute from the master mix tube to the white reaction well of the chip (Figure 16). *Avoid spillage of the clear solution outside the white reaction well. *Discard the pipette tip and master mix tube.

xiii. Click “YES” on the device screen to start the test. The PCR will be completed in 35 minutes.

xiv. Tap the “Open/Close Tray” button to eject the chip tray and discard the used chip immediately after the reaction.

xv. If MTB is detected test the same elute for RIF resistance using the Truenat MTB RIF Dx chip as a follow-on test. The test takes about 55 minutes.

**D. Running a RIF-Resistance Test**

i. If MTB is detected in a sample, Run a RIF resistance test.

iii. Use a portion of the same DNA eluate to test for RIF resistance using a Truenat MTB-RIF Dx chip.

iv. Start by returning to Step 3 in the PCR TB test process and repeat for RIFresistance by Selecting “MTB RIF” as the test type in the Truelab micro–PCR Analyser.

v. RIF-resistance testing takes an additional 60 minute.

***8.1.13 Biological Reference Interval*** Not applicable

***8.1.14 Interpretation and Reporting of Results***

**Interpretation** of results

At the end of the test run, the result screen will display;

i. “DETECTED” for Positive result.

ii. “NOT DETECTED” for Negative results. iii. MTB load as “HIGH”, “MEDIUM”, “LOW” or “VERY LOW” for positive.

iv. The result screen also displays the validity of the test run as “VALID” or “INVALID”.

NOTE: Invalid samples have to be repeated with fresh samples from the sample preparation stage.

IPC will co-amplify in most positive cases also, in some samples having a high target load, the IPC may not amplify, however, the test run is still considered valid Sample with MTB DETECTED should be tested for MTB RIF

**Reporting of results**

Click “VIEW RESULTS” on the menu bar. The View Results window appears.

Optional: Press “Print” to print the result page using Truelab® microPCR printer.

***Critical value***

MTB Detected RIF Resistance Detected

### *8.1.15 Limitation of the Procedure and Sources of Error*

i. Optimal performance of this test requires appropriate sample collection, handling, storage and transport to the test site.
ii. Though very rare, mutations within the highly conserved regions of the target genome where the Truenat™ assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
iii. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or samples. iv. A sample for which the Truenat™ assay reports “Not Detected” cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the Truenat™ assay should be interpreted in the context of other clinical and laboratory findings.
v. The performance of the test has not been evaluated with samples processed by methods other than those described in the package insert.
vi. Do not open the cartridge lid except when adding sample.
vii. Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
viii. Do not use a cartridge that has a damaged reaction tube. ix. Each single-use cartridge is used to process one test. Do not reuse spent cartridges.

### *8.1.16 Performance Characteristics*

Refer to the method verification report of this procedure.

***8.1.17 Supporting Document*** Sample collection manual

### *8.1.18 References*


- Truenat MTB Plus package insert version 5.
- The Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Device user manual.
- TBRL Bamenda Biosafety manual, Version 4.0, section 10.
- Truenat™-A Point-of-care Real Time PCR Test for Tuberculosis, video by Molbio available at https://youtu.be/ydR2I5S2v3


## 8.2 DIAGNOSIS OF MTB/RIF BY USING GENEXPERT SYSTEM

### *8.2.1 Purpose*

This procedure provides instructions for performing sample which is suspect with MTB/Rif

### *8.2.2 Scope*

This procedure is used for detection of the *Mycobacterium tuberculosis* complex bacteria and their rifampicin susceptibility using the Gene Xpert MTB/Rif system in microbiology section in the Laboratory.

### *8.2.3 Responsible*

Qualified, trained, Competent and Registered health laboratory personnel is responsible for ensuring the effective implementation for this procedure.

### *8.2.4 Principle*

The Gene Xpert MTB/RIF system is a fully automated nested real-time PCR system, which detects MTB complex DNA in smear positive and negative sputum samples and other body fluid i.e. pleural fluid, ascetic fluid CSF and Pus. It simultaneously identifies mutations in the rpoB gene, which are associated with rifampicin

resistance.

### *8.2.5 Sample Requirements*

- Collect minimum 1ml and maximum 4ml of sputum or other body fluid
- Do not accept samples with obvious food particles or other solid particulatesor blood stained (for this do Auramine O or ZN Stain)

### *8.2.6 6.0 Materials*

- MTB/RIF cartridges, Sample Reagent, Disinfectant solution (0.5% Jik and 70% alcohol), Sterile disposable transfer pipettes, Sterile screw-capped sample collection containers, Disposable gloves, Plastic bag for waste disposal, Labels and/or indelible labeling marker, Sterile pipettes for sample processing.

### *8.2.7 Equipment*

- Gene Xpert machine, Microscope, Personal Protective Equipment such as N95 respirator and Timer

### *8.2.8 Storage and stability*

- Sputum sample may be stored t 2-8 $^{o}$C before examinations.
- Protect the Sputumu samples from dierect sunlight.
- Store the Gene xpert catradges at 2-8$^{o}$C

### *8.2.9 Safety*

i. Decontaminate working surfaces twice daily, in the morning and afternoon

ii. Adhere to safety precautions as stated in the Safety manual

iii. All personal protective equipment (PPE) must be worn when performing this procedure.

iv. All samples must be regarded as potentially infections.

v. Avoid any contact between hands and eyes and nose during sample collection and testing.

vi. Do not use kit beyond the expiration date which appears on the package label or device in a damaged pouch.

vii. All spills should be wiped thoroughly using 1% sodium hypochlorite solution.

### *8.2.10 Calibration*

Calibarate auxillary equipment used in this operating this procedure once a year and kep record available is performed once per year.

### *8.2.11 Quality Control*

- The quality control will be performed on weekly bases (every week).
- Positive and negative known sample will be used.
- Ensure that all MTB/RIF cartridges and sample reagents used have passed the required are used within their expiry date

### *8.2.12 Procedure Steps*

***Start-up the Gene Xpert instrument and Preparation of sample***

i. Disinfect the working area by 0.5% jik.

ii. Label each Xpert MTB/RIF cartridge with the sample ID (e.g. NEW X_2016, HIV X_2016 or KID X_2016). Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge. Write on the sides of the cartridge or affix ID label.

iii. Leave sample in leak-proof sputum collection container.

iv. Unscrew lid of sputum collection container, add Sample Reagent 2:1 (v/v) to sample and close the lid again.

v. Shake vigorously 10 - 20 times.

vi. Incubate for 5 minutes at room temperature.

vii. Shake the sample again vigorously 10 – 20 times.

viii. Continue incubation for another 10 minutes.

***Note:*** *Samples should be liquefied with no visible clumps of sputum. If there are still clumps of sputum, shake again vigorously and incubate for another 35 min.*

### *8.2.13 Preparing the Cartridge*

- Start the test within 30 minutes of adding the sample to the cartridge

- Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark (= 2ml). Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge

NOTE: It is crucial that no bubbles are created when transferring the sample into the cartridge as this can lead to an error (no result).

Dispense slowly to minimize the risk of aerosol formation.

Close the cartridge lid. Make sure the lid snaps firmly into place.

Note: Remaining liquefied sample may be kept for up to 12 hrs at 2-8$^{o}$C should repeat testing be required.

### *8.2.14 Start the test on the GeneXpert instrument*

***Note: Before start processing the sample, check that the Gene Xpert instrument is functioning and the modules are available.***

I. Turn on the computer, and then turn on the Gene Xpert instrument.
II. On the Windows desktop, double-click the Gene Xpert shortcut icon.
III. Log on to the Gene Xpert System software using your user name and password.
IV. Click on “CHECK STATUS” and check if modules are available. If not proceed to “Troubleshooting” in User’s manual.
V. In the GeneXpertDx System window, click “CREATE TEST”. The Scan Cartridge Barcode dialog box appears.
VI. Scan the barcode on the Xpert MTB/RIF cartridge.
VII. The Create Test window appears.
VIII. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
IX. In the Sample ID box (ID=Patient names), scan or type the sample ID (e.g. NEW X_2016, HIV X_2016 or KID X_2016). Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the “View Results” window and all the reports X. Choose module.
XI. Click “Start Test”.
XII. In the dialog box that appears, type your password.
XIII. Open the instrument module door with the blinking green light and load the cartridge.
XIV. Close the door.
XV. The test starts and the green light stops blinking.
XVI. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
XVII. Dispose of used cartridges in the appropriate sample waste containers according to your institution’s standard practices

***8.2.15 Biological Reference interval*** Not Applicable

***Interpretation and Reporting of Results***

- MTB DETECTED, Rif Resistance DETECTED –RR
- MTB DETECTED, Rif Resistnce INDETEMINATE –TI
- MTB DETECTED Rif Not DETECTED – T
- MTB NOT DETECTED – N
- This is DNA based test, meant for **New TB suspect**, ensure don't enrol 'follow-up' patient.

The results are interpreted by the GeneXpert Dx system from measured fluorescent signals and embedded calculation algorithms and will be displayed in the **"View Results"** window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

**Critical values** MTB DETECTED, Rif Resistance DETECTED –RR

### *8.2.16 Limitation of the Procedure and Sources of Error*

- Perform the test and validate results as per this SOP and details of test package insert. Reliable results depend on proper sample collection, handling, and storage. A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB and rifampicin resistance. The results might be affected by antecedent or concurrent anti-TB drug therapy.

### *8.2.17 Performance Characteristics*

Refer to the verification report of Gene Xpert.

### *8.2.18 Supporting Document*

Sample collection manual and safety manual

### *8.2.19 References*


1. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C., USA, 2nd edition, 2007.
2. Monica cheesbrough (2005). District Laboratory Practice in Tropical countries. Cambridge University Press, New York, USA, 2nd edition, 2005.
3. WHO, (2003). Mannual of basic techniques for a health laboratory. Geneva. 2nd edition, 2003.
4. Manual of Clinical Microbiology. American Society for Microbiology (ASM), Washington D.C., USA. 9th edition, 2007.
5. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003.

6. International Union against Tuberculosis and Lung Disease. The Public Health Service National Tuberculosis Reference Laboratory and the National Laboratory Network. Paris; 1998.

## 8.3 DETERMINATION OF HIV -1 VIRAL LOAD BY USING GENEXPERT SYSTEM

### *8.3.1 Purpose*

This SOP outlines the steps for qualitative *in vitro* diagnostic HVL test by using automated GeneXpert system.

### *8.3.2 Scope*

The Xpert HIV-1 VL assay is an *in vitro* reverse transcriptase polymerase chain reaction (RT-PCR) assay for the detection and quantification of Human Immunodeficiency Virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals, using the automated GeneXpert Instrument Systems. The assay can quantify HIV-1 RNA over the range of 40 to 10,000,000copies/mL. The Xpert HIV-1 VL assay is validated for quantification of RNA from HIV-1 Group M (subtypes A, B, C, D, F, G, H, J, K, CRF01_AE, CRF02_AG, and CRF03_AB), Group N, and Group O.

The Xpert HIV-1 VL assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels.

The Xpert HIV-1 VL assay is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection

### *8.3.3 Responsible*

The Qualified, competent and registered health laboratory practitioners are responsible to carry out this procedure.

The head of section is responsible for ensuring the effective implementation and competency assessment for this procedure

### *8.3.4 Principles Principle of the Procedure*

GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex specimens using real-time reverse transcriptase PCR (RT-PCR). The systems consist of an instrument, personal computer, and preloaded software for

running tests and viewing the results. The systems require single-use disposable GeneXpert cartridges that contain the RT-PCR reagents and carry out the sample extraction and RT-PCR processes. Because the cartridges are self-contained, crosscontamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx Operator Manual* or *GeneXpert Infinity Operator* **Manual.**

The HIV-1 VL assay includes reagents for the detection of HIV-1 RNA in specimens and two internal controls used for quantitation of HIV-1 RNA. The internal controls are also used to monitor the presence of inhibitor(s) in the RT and PCR reactions.

The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability

***Principle of operation***

Each GeneXpert Dx module processes one sample. You insert the sample and applicable reagents into a GeneXpert cartridge, create a test, load the cartridge into an available instrument module, and then start the test. During the test, the system performs the following steps:

i. Moves the sample and reagents into different chambers in the cartridge for sample preparation.
ii. Hydrates the reagent beads. iii. Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step).
iv. Moves the sample and reagent mixture into the reaction tube.
v. Starts the PCR cycles and real-time detection.

### *8.3.5 Sample Requirement.*

- DBS collected as per SOP for collection of DBS.
- Anticoagulated whole blood(WB) B in sterile tubes using EDTA (lavender top) as the anticoagulant as per the manufacturer's intructions for use.
- A minimum of 100 µL of WB is required for the HIV-1 Qualitative assay.

### *8.3.6 Equipment*

Perform equipment Start up, Maintenance, trouble shoot and shut down refer manufacturer instructions.

Biosafety cabinet, Data computer connected to LIS (Optional), Printer (optional), GeneXpert instrument and GeneXpert Software should be available.

### *8.3.7 Materials*

| Reagent kit content | Extra supplies |
|---|---|

| | |
|---|---|
| The HIV-1 Qual assay kit contains sufficient reagents to process 10 specimens or quality control samples.<br>The kit contains the following:<br>HIV-1 Qual assay Cartridges with Integrated Reaction Tubes 10<br>• Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge<br>• Lysis Reagent (Guanidinium Thiocyanate) 1.4 mL per cartridge<br>• Rinse Reagent 0.5 mL per cartridge<br>• Elution Reagent 2.5 mL per cartridge<br>• Binding Reagent 2.4 mL per cartridge<br>• Proteinase K Reagent 0.48 mL per cartridge | • Serviette/Wipe<br>• Bleach<br>• 70 % alcohol or methylated spirit<br>• Labelling marker<br>• Optional: sterile pipettes for sample processing<br>• Lab coat,<br>• Non - powdered gloves |
| HIV-1 Qual assay Sample Reagent Set (Sample Reagent) 10<br>• Lysis Reagent (Guanidinium Thiocyanate) 1.0 mL per vial Disposable 1 mL Transfer Pipettes 1 bag of 10 per kit<br>Disposable 100 µL Transfer Micropipettes 1 bag of 10 per kit<br>**CD 1 per kit**<br>• Assay Definition Files (ADF)<br>• Instructions to import ADF into GeneXpert software<br>• Instructions for Use (Package Insert) | |

### *8.3.8 Storage and stability*

***Reagents***

- Store the HIV-1 Qualitative assay cartridges and reagents at 2–28 °C.
- Do not use any reagents that have become cloudy or discoloured.
- Do not use a cartridge that has leaked.
- Use cartridge within 30 minutes after adding the sample
- Reagents are stable until their expiration dates when stored and handled as per instruction for use.

***Samples***

- DBS cards may be stored at 18–30 °C for 30 days or – 15°C - 20 °C or colder for up to 4 months, or -70 °C for longer storage.
- EDTA-anticoagulated WB may be stored at 31–35 °C for up to 8 hours, 15–30 °C for up to 24 hours or at 2–8 °C for up to 72 hours, prior to sample preparing and testing

### *8.3.9 Safety*

i. Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents.

ii. Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
iii. Follow safety procedures for working with chemicals and handling biological samples.
iv. When processing more than one sample at a time, open only one cartridge; add sample and close the cartridge before processing the next sample. Change gloves between samples.
v. Do not substitute HIV-1 Qual assay reagents with other reagents.
vi. Do not open the HIV-1 Qual assay cartridge lid except when adding the Sample Reagent and WB or the Sample Reagent treated DBS sample.
vii. Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
viii. Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
ix. Do not use a cartridge that has a damaged reaction tube.
x. Each single-use HIV-1 Qual assay cartridge is used to process one specimen. Do not reuse spent cartridges. xi. The single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
xii. In the event of contamination of the work area or equipment with specimen or control materials, disinfect the area with a 1:10 bleach solution and then 70% ethanol. Wipe work surfaces dry completely before proceeding.

### *8.3.10 Calibration*

There is no need to calibrate the GeneXpert Dx instrument. Cepheid performs all of the necessary calibrations before you receive the system. However, Cepheid recommends that the instrument be recalibrated after 1 year of use, based on the initial installation date (or based on the previous calibration for subsequent years) or at 2000 tests per instrument module, whichever comes first.

### *8.3.11 Quality control*

The GeneXpert Dx System automatically performs internal quality control for each sample. During each test, the system uses one or more of the following controls: **Internal control (IC**)—Verifies the performance of the PCR reagents and prevents a false negative result. The internal control PCR assay assesses if there is any inhibition, possibly by components, in the test sample. The internal control is provided in the cartridge and should be positive in a negative sample.

**Endogenous control (EC)**—Normalizes targets and ensures sufficient sample is used in the test. Because of its low variability, the endogenous control can also be

used to indicate sample-inhibitor contamination. The endogenous control is taken from the specimen sample.

Each test includes a Sample Volume Adequacy (SVA), a Sample Processing Control (SPC) and Probe Check Control (PCC).

**Sample Volume Adequacy (SVA)**: Ensures that the sample was correctly added to the cartridge. The SVA verifies that the correct volume of sample has been added in the sample chamber. The SVA passes if it meets the validated acceptance criteria. If the SVA does not pass, an ERROR 2096 will display if there is no sample or an ERROR 2097 if there is not enough sample. The system will prevent the user from resuming the test.

**Sample Processing Control (SPC)**: Ensures that the sample was correctly processed. The SPC is an Armoured RNA in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample virus. The SPC verifies that lysis of HIV-1 has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally, this control detects specimenassociated inhibition of the RT-PCR reaction. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

In addition to the controls, the GeneXpert Dx instrument performs a probe check during the first stage of the test.

**Probe Check Control (PCC)**: Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

**External Controls**: Internal quality controls should be done weekly by using known HIV-1 DETECTED and HIV -1 NOT DETECTED as the same as the routine EID/DBS samples.

### *8.3.12 Procedural steps*

Follow the actions described step by step to do each specific task

***Start-up the GeneXpert instrument***

- Turn on the GeneXpert Dx instrument, and then turn on the computer.
- On the Windows desktop, double-click the GeneXpert Dx shortcut icon.
- Log on to the GeneXpert Dx System software using your user name and password.
- Click on “CHECK STATUS” and check if modules are available. If not proceed to “Troubleshooting” in User manual.

**! Note:** Before start processing the specimen, check that the GeneXpert instrument is functioning and the modules are available.

***Preparing of cartridge(s)***

***EDTA anticoagulated Whole Blood***

i. Wear protective disposable gloves. ii. Disinfect the work area by 0.5 % bleach solution followed by 70 % alcohol

iii. Wear protective disposable powder free gloves.

iv. Label the Sample Reagent vial with the specimen identification.

v. Inspect the test cartridge for damage. If damaged, do not use. vi. Open the cartridge lid.

vii. Use the 1 mL transfer pipette provided to transfer 750 µL of the sample reagent into the sample chamber of the cartridge.

viii. Allow the Sample Reagent to adjust to room temperature and mix the bottle by inverting before transferring to the cartridge. Transfer exactly 750 µL into the sample chamber of the cartridge.

ix. Mix the Whole Blood sample by inverting the vial (EDTA or lavendertop tube) at least seven times. Immediately transfer 100 µL using the micropipette provided by squeezing the upper bulb and then releasing to aspirate the blood.

x. Squeeze again to dispense the blood into the sample chamber of the cartridge where it will mix with the Sample Reagent already in the sample chamber. Alternatively, use an automatic pipette to dispense the blood into the sample chamber of the cartridge. Do **NOT** pour the specimen into the chamber!

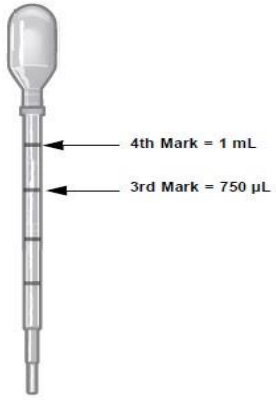



*Figure 1. HIV-1 Qual Assay 1 mL Transfer Pipette*

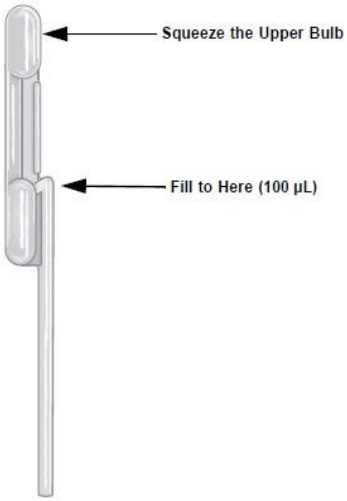



*Figure 2. HIV-1 Qual Assay 100 µL Transfer Micropipette*

*Figure 3. HIV-1 Qual Assay Cartridge (Top View)*

❑ ***DBS sample***

i. Wear protective disposable gloves.

ii. Disinfect the working area. iii. Wear protective disposable powder free gloves. iv. Before starting, remove the vial containing the Sample Reagent from the kit and, if it was refrigerated, allow to adjust to room temperature. If the vial has not been stored in an upright position, make sure the buffer is settled in the bottom by giving the vial a firm shake.

v. Turn on Thermo Mixer to heat to 56 °C.

vi. Label the Sample Reagent vial with the specimen identification.

vii. Using sterilized scissors, excise one entire DBS from the filter paper card for each specimen. Follow the delineated lines when excising the DBS. If perforated circles are used, use clean and sterile pipette tips to detach the DBS.

viii. Unscrew the lid on the vial containing the Sample Reagent and place one DBS in the vial. Ensure that the DBS is fully submerged in the Sample Reagent buffer.

ix. Place the vial with the DBS in a Thermo Mixer and incubate for 15 minutes at 56 °C while rotating at 500 rpm.

x. Inspect the test cartridge for damage. If damaged, do not use. xi. Open the cartridge lid

xii. Use the 1 mL transfer pipette provided to transfer all the liquid from the lysed DBS specimen into the sample chamber of the cartridge. Ensure the pipette is filled above the third mark on the transfer pipette. Avoid suction of the DBS with the pipette. Do **NOT** pour the specimen into the chamber!

xiii. Close the cartridge lid, ready to start the test.

**Notes! Change gloves between specimen, and each new procedure.**

***Starting the Test***

i. In the GeneXpert System window, click **Create Test.** The scan Cartridge Barcode dialog box appears.

ii. Scan the barcode on the HIV-1 Qual assay cartridge. iii. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

iv. Type the Patient ID, make sure the Patient ID is typed correctly.

v. Type in the **Sample ID**. Make sure the Sample ID is typed correctly.

vi. On the **Notes** field, enter the words **KATAVI RRHL** to indicate a testing laboratory name on the patient report.

vii. Open the instrument module door with the blinking green light and load the cartridge.

viii. Click **Start Test** (Gene Xpert Dx). Enter you're your user name and password, if requested.

ix. Close the door.

x. The test starts and the green light stops blinking. When the test is finished, the light turns off. xi. Wait until the system releases the door lock before opening the module door and removing the cartridge.

***Result viewing, and printing***

i. In the Gene Xpert Dx System window, Click the **View Results** icon to view results. This view result window appears.

ii. If the software reports "Error", Invalid, or No result, repeat the test using new DBS circle.

iii. Should the test again show Error, Invalid, or No result, proceed to troubleshooting manual to exclude technical problems before requesting a new specimen.

iv. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

v. Report should be done in the Laboratory HEID register and a tick on the respective result blank space on the HEID request form. Report as; HIV -1 DETECTED*.
HIV -1 NOT DETECTED

vi. Report “please submit a new sample” if the system repeatedly did not produce a result and you have excluded and/or fixed a technical problem.
vii. HIV -1 DETECTED is a critical result that needs immediate action including but not limited to; retesting using new DBS circle with a new cartridge, and result notification to the respective referring health facility.

***8.3.13 Biological Reference Intervals*** Not Applicable

***8.3.14 15. Result interpretation and Reporting of Results.***

• The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. Possible results are shown in Table below:

| **Result** | **Interpretation** |
|---|---|
| **HIV-1 DETECTED** See Figure 1. | The HIV-1 target nucleic acids are detected.<br>• The HIV-1 target nucleic acids have a Ct within the valid range.<br>• SPC: NA (not applicable); SPC is ignored because the HIV-1 target amplification occurred.<br>• Probe Check: PASS; all probe check results pass. |
| **HIV-1 NOT DETECTED** See Figure 2. | The HIV-1 target nucleic acids are not detected. SPC meets acceptance criteria.<br>• SPC: PASS; SPC has a Ct within the valid range.<br>• Probe Check: PASS; all probe check results pass. |
| **INVALID** | Presence or absence of the HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>• SPC: FAIL; SPC Ct is not within valid range.<br>• Probe Check: PASS; all probe check results pass |
| **ERROR** | Presence or absence of HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>• HIV-1: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: FAIL*; all or one of the probe check results fail.<br>*If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. |

| | |
|---|---|
| **NO RESULT** | Presence or absence of HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.<br>• HIV-1: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: NA (not applicable). |

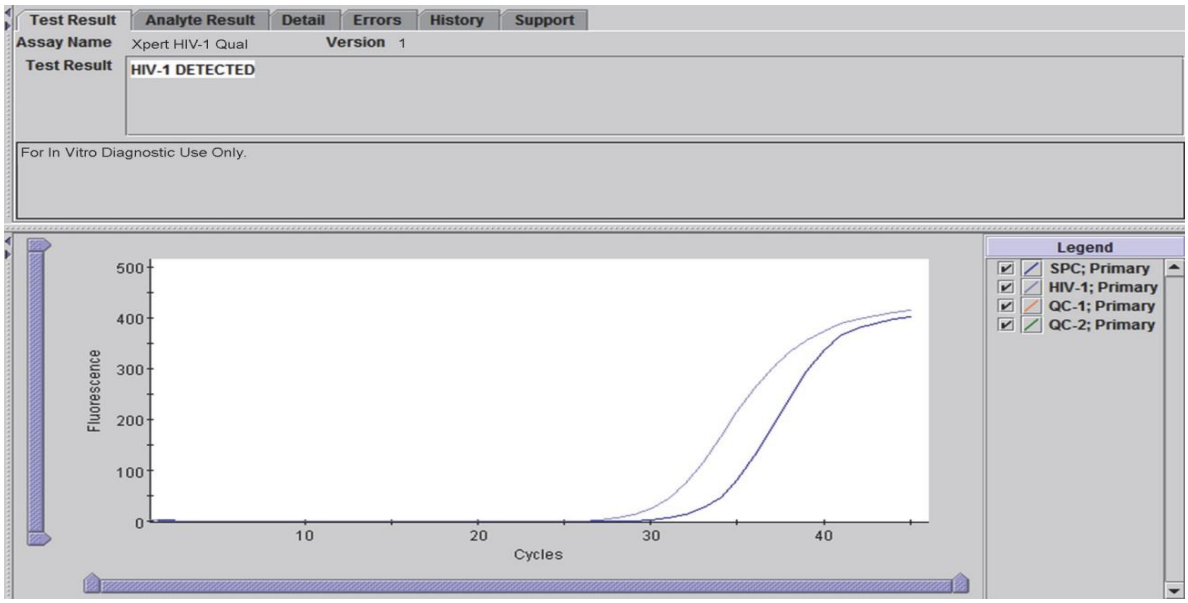



***Figure 4 HIV -1 DETECTED***

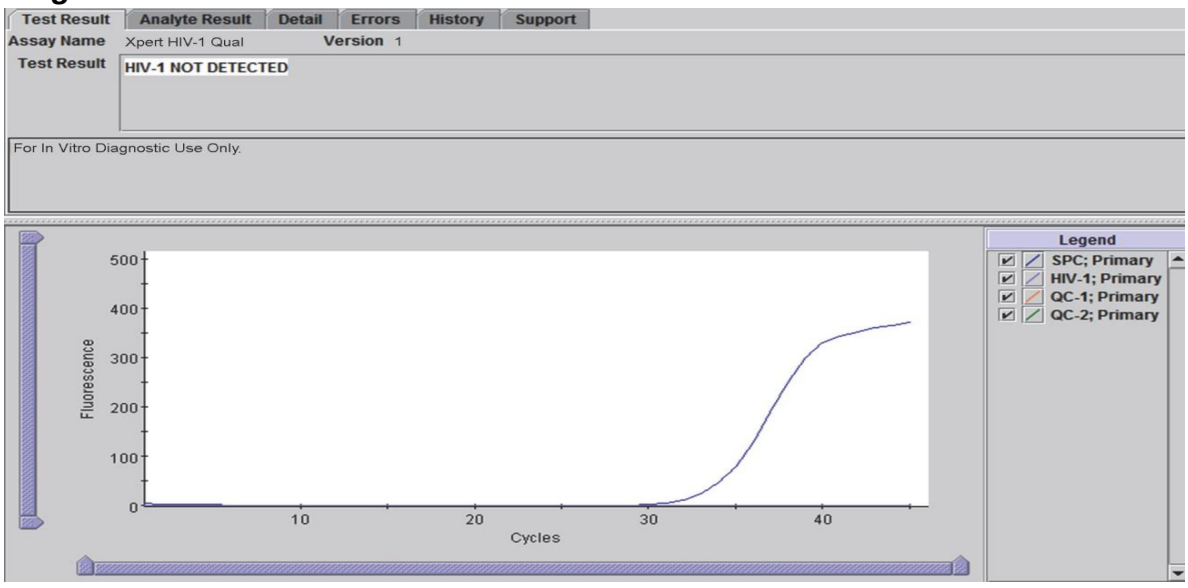



***Figure 5. HIV -1 NOT DETECTED***

### *8.3.15 Limitation of the Procedure and Sources of Error*

Good laboratory practices and changing gloves between handling specimens are recommended to avoid contamination of reagents.

Rare mutations within the target region of the HIV-1 Qual assay may affect primer and/or probe binding resulting in failure to detect the virus.

A negative test result does not preclude HIV-1 infection. Results from the HIV-1 Qual assay should be interpreted in conjunction with clinical presentation and other laboratory markers.

### *8.3.16 Performance Characteristics*

Refer to the manufacture package insert and verification report for detailed information on Performance Characteristics of the testing procedure

### *8.3.17 Supporting Documents*

- Sample collection manual and safety manual
- Quality manual

### *8.3.18 References*

- Ministry of Health, Community Development, Gender, Elderly and Children, Standard Operating Procedures for qualitative HIV-1 HEID testing using GeneXpert
- Xpert HIV-1 Qual -1 Assay Package Insert 308-3048 Rev J
- GeneXpert Dx System. Operator Manual

## 8.4 DETERMINATION OF HIV EARLY INFANT DIAGNOSIS BY USING GENEXPERT SYSTEM

### *8.4.1 Purpose*

This SOP outlines the steps for qualitative *in vitro* diagnostic HIV-1 test by using automated GeneXpert system.

### *8.4.2 Scope*

The HIV-1 Qual assay, is a qualitative *in vitro* diagnostic test designed to detect Human Immunodeficiency Virus Type 1 (HIV-1) total nucleic acids on the automated Gene Xpert Systems using human whole blood (WB) and dried blood spot (DBS) specimens from individuals suspected of HIV-1 infection. The HIV-1 Qualitative assay is intended to aid in the diagnosis of HIV-1 infection in conjunction with clinical presentation and other laboratory markers. The assay is intended to be used by laboratory professionals or specifically-trained healthcare workers. The assay is not intended to be used as a blood donor screening test for HIV-1.

### *8.4.3 Responsible*

Section head is responsible effective implementation of this procedure. Only competent laboratory staffs should carry out this procedure. It is the responsibility of each staff to read, understand and implement this procedure.

### *8.4.4 Principle*

***Principle of the Procedure***

The GeneXpert (GX) Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real time reverse transcription PCR (RT-PCR). The systems consist of an instrument, personal computer, and preloaded software for performing tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR reagents and host the RT-PCR processes. Because the cartridges are self-contained, crosscontamination between samples is minimized. The HIV-1 Qual assay includes reagents for the detection of HIV-1 total nucleic acids in specimens as well as an internal control to ensure adequate processing of the target and to monitor the presence of inhibitor(s) in the RT and PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

### *8.4.5 Principle of operation*

Each GeneXpert Dx module processes one sample. You insert the sample and applicable reagents into a GeneXpert cartridge, create a test, load the cartridge into an available instrument module, and then start the test. During the test, the system performs the following steps:

i. Moves the sample and reagents into different chambers in the cartridge for sample preparation.
ii. Hydrates the reagent beads. iii. Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step).
iv. Moves the sample and reagent mixture into the reaction tube.
v. Starts the PCR cycles and real-time detection.

### *8.4.6 Sample requirements*

DBS collected as per SOP for collection of DBS.

EDTA Anticoagulated WB in sterile tubes using EDTA (lavender top) as the anticoagulant as per the manufacturer's instructions for use. A minimum of 100 µL of WB is required for the HIV-1 Qualitative assay.

### *8.4.7 Equipment*

Start up, Maintainance, trouble shoot and shud down refer manaufacturer instructions Thermo Mixer C for incubation with smart block, Biosafety cabinet, Data computer connected to LIS (Optional) and Printer (optional)

### *8.4.8 Materials*

Materials (Reagents and consumables) used to perform the test.

| Reagent kit content | Extra consumables |
|---|---|
| The HIV-1 Qual assay kit contains sufficient reagents to process 10 specimens or quality control samples.<br>The kit contains the following:<br>HIV-1 Qual assay Cartridges with Integrated Reaction Tubes 10<br>• Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge<br>• Lysis Reagent (Guanidinium Thiocyanate) 1.4 mL per cartridge<br>• Rinse Reagent 0.5 mL per cartridge<br>• Elution Reagent 2.5 mL per cartridge<br>• Binding Reagent 2.4 mL per cartridge<br>• Proteinase K Reagent 0.48 mL per cartridge<br>HIV-1 Qual assay Sample Reagent Set (Sample Reagent) 10<br>• Lysis Reagent (Guanidinium Thiocyanate) 1.0 mL per vial Disposable 1 mL Transfer Pipettes 1 bag of 10 per kit<br>Disposable 100 µL Transfer Micropipettes 1 bag of 10 per kit **CD 1 per kit**<br>• Assay Definition Files (ADF)<br>• Instructions to import ADF into GeneXpert software<br>• Instructions for Use (Package Insert) | • DBS Collection Kit (Filter paper cards, e.g., Whatman 903, Munktell or equivalent, lancets, desiccants, plastic<br>• sealable bags, and swabs)<br>• Scissors, sterile (recommended for excising DBS from filter paper if not using a perforated DBS card)<br>• Sterile pipette tips<br>• Serviette/Wipe<br>• Bleach<br>• 70 % alcohol or methylated spirit<br>• Distilled water<br>• Labelling marker<br>• Optional: sterile pipettes for sample processing |

| | |
|---|---|
| | • Lab coat,<br>• Non - powdered gloves |

### *8.4.9 Storage and Stability.*

DBS cards may be stored at 18–30 °C for 30 days or – 15°C - 20 °C or colder for up to 4 months, or -70 °C for longer storage.

EDTA-anticoagulated WB may be stored at 31–35 °C for up to 8 hours, 15–30 °C for up to 24 hours or at 2–8 °C for up to 72 hours, prior to preparing and testing the specimen.

### *8.4.10 Safety*

i. Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents.
ii. Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
iii. Follow safety procedures for working with chemicals and handling biological samples.
iv. When processing more than one sample at a time, open only one cartridge; add sample and close the cartridge before processing the next sample. Change gloves between samples.
v. Do not substitute HIV-1 Qual assay reagents with other reagents.
vi. Do not open the HIV-1 Qual assay cartridge lid except when adding the Sample Reagent and WB or the Sample Reagent treated DBS sample.
vii. Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
viii. Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
ix. Do not use a cartridge that has a damaged reaction tube.
x. Each single-use HIV-1 Qual assay cartridge is used to process one specimen. Do not reuse spent cartridges. xi. The single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
xii. In the event of contamination of the work area or equipment with specimen or control materials, disinfect the area with a 1:10 bleach solution and then 70% ethanol. Wipe work surfaces dry completely before proceeding.

### *8.4.11 Calibration*

You do not need to calibrate the GeneXpert Dx instrument. Cepheid performs all of the necessary calibrations before you receive the system. However, Cepheid

recommends that the instrument be recalibrated after 1 year of use, based on the initial installation date (or based on the previous calibration for subsequent years) or at 2000 tests per instrument module, whichever comes first.

### *8.4.12 Quality control*

Quality control is an important part of *in vitro* diagnostic testing because it ensures you are performing the tests correctly and that your GeneXpert Dx System is working properly. The GeneXpert Dx System automatically performs internal quality control for each sample. During each test, the system uses one or more of the following controls:

- **Internal control (IC**)—Verifies the performance of the PCR reagents and prevents a false negative result. The internal control PCR assay assesses if there is any inhibition, possibly by components, in the test sample. The internal control is provided in the cartridge and should be positive in a negative sample.
- **Endogenous control (EC)**—Normalizes targets and ensures sufficient sample is used in the test. Because of its low variability, the endogenous control can also be used to indicate sample-inhibitor contamination. The endogenous control is taken from the specimen sample.

Each test includes a Sample Volume Adequacy (SVA), a Sample Processing Control (SPC) and Probe Check Control (PCC).

**Sample Volume Adequacy (SVA)**: Ensures that the sample was correctly added to the cartridge. The SVA verifies that the correct volume of sample has been added in the sample chamber. The SVA passes if it meets the validated acceptance criteria. If the SVA does not pass, an ERROR 2096 will display if there is no sample or an ERROR 2097 if there is not enough sample. The system will prevent the user from resuming the test.

**Sample Processing Control (SPC)**: Ensures that the sample was correctly processed. The SPC is an Armoured RNA in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample virus. The SPC verifies that lysis of HIV-1 has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally, this control detects specimenassociated inhibition of the RT-PCR reaction. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

In addition to the controls, the GeneXpert Dx instrument performs a probe check during the first stage of the test.

**Probe Check Control (PCC)**: Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor

bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

**External Controls**: Internal quality controls should be done weekly by using known HIV-1 DETECTED and HIV -1 NOT DETECTED as the same as the routine EID/DBS samples.

### *8.4.13 Procedural steps*

Follow the actions described step by step to do each specific task

#### *Start-up the GeneXpert instrument*

- Turn on the GeneXpert Dx instrument, and then turn on the computer.
- On the Windows desktop, double-click the GeneXpert Dx shortcut icon.
- Log on to the GeneXpert Dx System software using your user name and password.
- Click on “CHECK STATUS” and check if modules are available. If not proceed to “Troubleshooting” in User manual.

***Note: Before start processing the specimen, check that the GeneXpert instrument is functioning and the modules are available.***

#### *Preparing of cartridge(s) for EDTA anticoagulated Whole Blood*

i. Wear protective disposable gloves.

ii. Disinfect the work area by 0.5 % bleach solution followed by 70 % alcohol

iii. Wear protective disposable powder free gloves. iv. Label the Sample Reagent vial with the specimen identification.

v. Inspect the test cartridge for damage. If damaged, do not use. vi. Open the cartridge lid.

vii. Use the 1 mL transfer pipette provided to transfer 750 µL of the sample reagent into the sample chamber of the cartridge.

viii. Allow the Sample Reagent to adjust to room temperature and mix the bottle by inverting before transferring to the cartridge. Transfer exactly 750 µL into the sample chamber of the cartridge. ix. Mix the Whole Blood sample by inverting the vial (EDTA or lavender-top tube) at least seven times. Immediately transfer 100 µL using the micropipette provided by squeezing the upper bulb and then releasing to aspirate the blood.

x. Squeeze again to dispense the blood into the sample chamber of the cartridge where it will mix with the Sample Reagent already in the sample chamber. Alternatively, use an automatic pipette to dispense the blood into the sample chamber of the cartridge. Do **NOT** pour the specimen into the chamber!

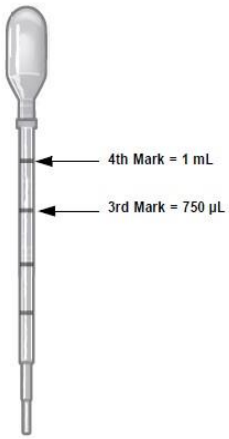



*Figure 6. HIV-1 Qual Assay 1 mL Transfer Pipette*

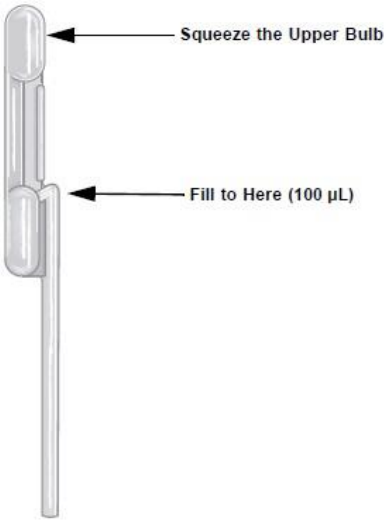



*Figure 7. HIV-1 Qual Assay 100 µL Transfer Micropipette*

*Figure 8. HIV-1 Qual Assay Cartridge (Top View)*

***DBS sample***

i. Wear protective disposable gloves.

ii. Disinfect the working area. iii. Wear protective disposable powder free gloves. iv. Before starting, remove the vial containing the Sample Reagent from the kit and, if it was refrigerated, allow to adjust to room temperature. If the vial has not been stored in an upright position, make sure the buffer is settled in the bottom by giving the vial a firm shake.

v. Turn on Thermo Mixer to heat to 56 °C.

vi. Label the Sample Reagent vial with the specimen identification. vii. Using sterilized scissors, excise one entire DBS from the filter paper card for each specimen. Follow the delineated lines when excising the DBS. If perforated circles are used, use clean and sterile pipette tips to detach the DBS.

viii. Unscrew the lid on the vial containing the Sample Reagent and place one DBS in the vial. Ensure that the DBS is fully submerged in the Sample Reagent buffer.

ix. Place the vial with the DBS in a Thermo Mixer and incubate for 15 minutes at 56 °C while rotating at 500 rpm.

x. Inspect the test cartridge for damage. If damaged, do not use.

xi. Open the cartridge lid

xii. Use the 1 mL transfer pipette provided to transfer all the liquid from the lysed DBS specimen into the sample chamber of the cartridge. Ensure the pipette is filled above the third mark on the transfer pipette. Avoid suction of the DBS with the pipette. Do **NOT** pour the specimen into the chamber!

xiii. Close the cartridge lid, ready to start the test.

***Notes Change gloves between specimen, and each new procedure.***

### *Starting the Test*

i. In the GeneXpert System window, click **Create Test.** The scan Cartridge Barcode dialog box appears.

ii. Scan the barcode on the HIV-1 Qual assay cartridge.

iii. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

iv. Type the Patient ID, make sure the Patient ID is typed correctly.

v. Type in the **Sample ID**. Make sure the Sample ID is typed correctly.

vi. Open the instrument module door with the blinking green light and load the cartridge.

vii. Click **Start Test** (Gene Xpert Dx). Enter you're your user name and password, if requested.

viii. Close the door. ix. The test starts and the green light stops blinking. When the test is finished, the light turns off.

x. Wait until the system releases the door lock before opening the module door and removing the cartridge.

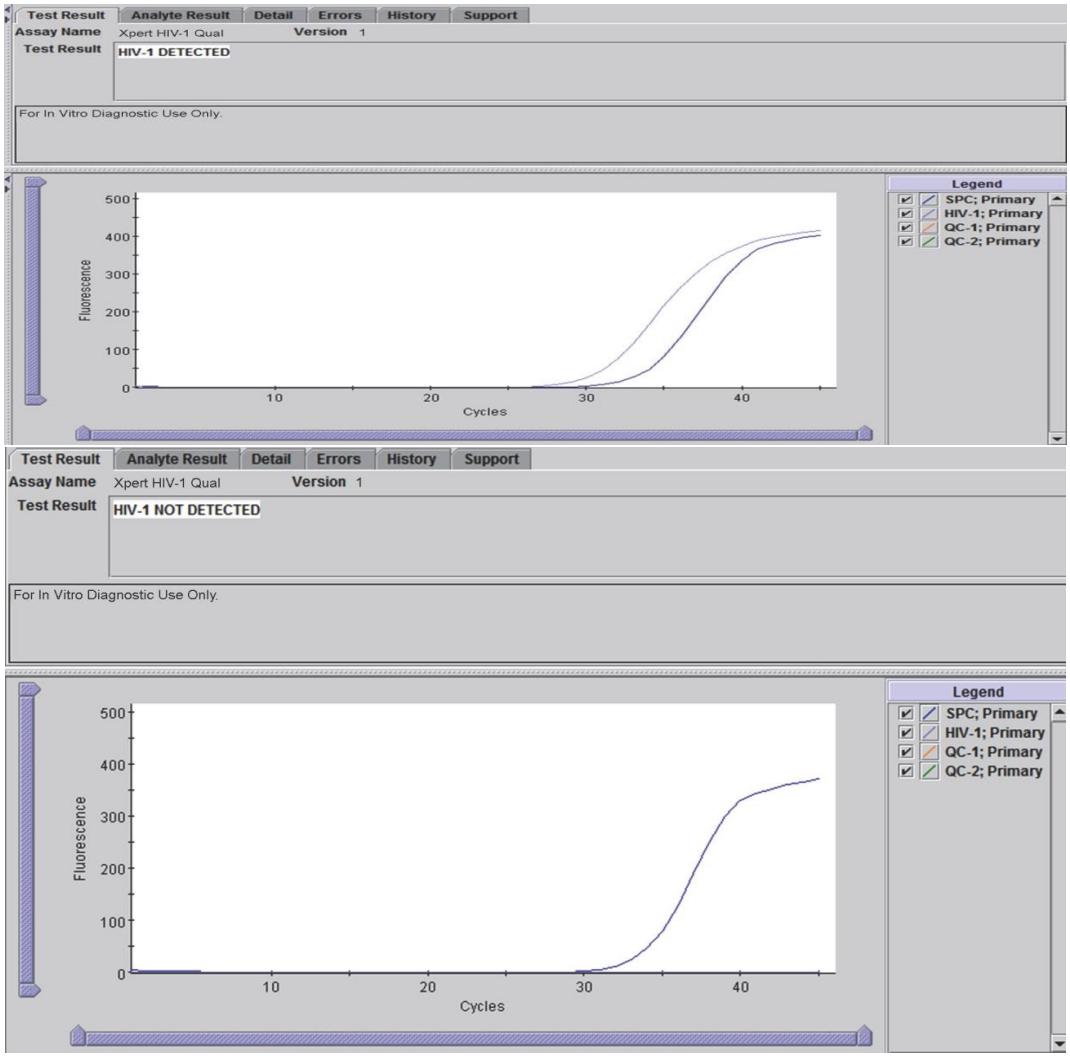
### *8.4.14 Biological Reference Intervals.*

Not Applicable.

### *8.4.15 Interpretation and reporting of Results*

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. Possible results are shown in Table below:

| Result | Interpretation |
|---|---|
| **HIV-1 DETECTED**<br>See Figure 1. | The HIV-1 target nucleic acids are detected.<br>• The HIV-1 target nucleic acids have a Ct within the valid range.<br>• SPC: NA (not applicable); SPC is ignored because the HIV-1 target amplification occurred.<br>• Probe Check: PASS; all probe check results pass. |
| **HIV-1 NOT DETECTED**<br>See Figure 2. | The HIV-1 target nucleic acids are not detected. SPC meets acceptance criteria.<br>• SPC: PASS; SPC has a Ct within the valid range.<br>• Probe Check: PASS; all probe check results pass. |
| **INVALID** | Presence or absence of the HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>• SPC: FAIL; SPC Ct is not within valid range.<br>• Probe Check: PASS; all probe check results pass |
| **ERROR** | Presence or absence of HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>• HIV-1: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: FAIL*; all or one of the probe check results fail. *If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. |
| **NO RESULT** | Presence or absence of HIV-1 target nucleic acids cannot be determined. Repeat test with new sample and cartridge.<br>A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.<br>• HIV-1: NO RESULT<br>• SPC: NO RESULT<br>• Probe Check: NA (not applicable). |




### *8.4.16 Limitation of the Procedure and Sources of Error.*

Good laboratory practices and changing gloves between handling specimens are recommended to avoid contamination of reagents.

Rare mutations within the target region of the HIV-1 Qual assay may affect primer and/or probe binding resulting in failure to detect the virus.

A negative test result does not preclude HIV-1 infection. Results from the HIV-1 Qual assay should be interpreted in conjunction with clinical presentation and other laboratory markers

### *8.4.17 Performance Characteristics*

Refer to the manufacture package insert for detailed information on Performance Characteristics of the testing procedure.

### *8.4.18 Supporting Documents*

Sample collection manual, Quality manual

### *8.4.19 References*

Ministry of Health, Community Development, Gender, Elderly and Children, Standard Operating Procedures for qualitative HIV-1 HEID testing using GeneXpert

Xpert HIV-1 Qual -1 Assay Package Insert 308-3048 Rev J

GeneXpert Dx System. Operator Manual

# CHAPTER NINE: ANATOMICAL PATHOLOGY

## 9.1 PROCEDURE FOR MORTUARY SERVICES

### *9.1.1 Purpose*

This procedure provides instructions for providing mortuary services including autopsy practice, embalming as well as safety of personnel, visitors and community.

### *9.1.2 Scope*

This document provides guidelines for the mortuary staff and administration recommended standards for mortuary facilities in settings and for communication between staff involved in autopsy procedures or autopsy related processes.

Body storage

A body cold store having a capacity appropriate for the mortuary workload should be maintained at a temperature of about 2- 6 $^{o}$C.

If long term storage is required, the body should have maintained at approximately 20 $^{o}$C.

Labelling procedures should be established so that body identification is made easy.

### *9.1.3 Responsible*

Qualified and trained mortuary personnel are responsible for implementing this procedure.

The Head of Laboratory is responsible for ensuring the effective implementation and maintenance of this procedure.

***9.1.4 Principle*** Not applicable

***9.1.5 Sample requirements*** Not applicable

### *9.1.6 Equipment*

Refrigerator, Trolley, Post-mortem kit

### *9.1.7 Materials*

10% formalin, Gloves, Leak proof bag, gloves, water resistant gown/plastic apron over water repellent gown, and surgical masks, goggles or face shield, shoe covers

### *9.1.8 Storage and stability*

Dead body: For short term store at 2-6$^{o}$C, For long term store -20$^{o}$C

10% formalin: Store at room temperature

### *9.1.9 Safety*

- PPE shall be used to prevent skin and mucous membrane contact with blood and other body fluid. These may include the use of gloves, N95 masks, protective eye wear, face shields, shoe covers, plastic aprons/gowns hair bornets, cut resistant gloves and laboratory coats.
  Surgical or post mortem gloves must be worn by all personnel involved in the autopsy procedure **Hand washing:**
- Hand and other skin surface should be washed with soaps and water immediately after contact with blood or other body fluid. Hand shall be washed each time with running water and soap.
- Sufficient and appropriate disinfectant should be 0.5% chlorine solution for routine mortuary work, embalming and post-mortem, then removed and rinsed with distilled water before being dried and stored.
- Appropriate vaccination and follow up of immunity status should be offered to all mortuary staff and record should be maintained including any refusal of an offer of immunization.
- The Mortuary personnel is responsible to ensuring that he/she complies with policies guarding personal safety, handling of bodies and bodily fluids as well as the safe operation of Mortuary equipment.
- Put bio hazardous waste in a red biohazard bag
- Put the red bag in the bio hazardous waste box
- Tape box closed
- Put taped box in bio hazardous waste pick up location
- The Medical Attendants must wear complete PPE when handling hazardous waste.

  **Autopsy Instruments:**
- All instruments are to be cleaned and disinfected between examinations, instruments should be dried and laid out on a non-metal surface
- Autopsy Tables and Garbage Disposals: Autopsy tables should be cleaned and disinfected between autopsies or at the end of the day
- Following the autopsy, the disposals and drain on each autopsy table should be cleaned.

### *9.1.10 Calibration*

Calibration for refrigerators should be done as per schedule

***9.1.11 Quality control*** Not applicable

### *9.1.12 Procedure for receiving died body from inside the facility.*

After receiving a call from the ward there is a dead body the following procedure should be followed.

- Prepare the trolley by making sure it is well covered.
- Make sure you have put on proper personal protective equipment such as apron, face mask, boot and gloves.

### *9.1.13 In the ward you should observe the following before:*

If the dead body is well labelled

> If the mortuary forms have been filled in and signed by the nurse

>Make sure you sign the death book before you take the body

### *9.1.14 Once you reach in the mortuary do the documentation first*

- inform the relatives about the process that they should follow including how to do the payments and how to get the discharge summary.
- Dead body should be kept in a cooling unit (with tags as above)
- Make sure to label on the form the number of the fridge (unit) and the fridge should have a tag number which should match with that attached to the body inside.

Note: Tags have to be attached to the big toes /or around the circumference of the ankle

***Receiving dead body from outside the facility brought by police, relatives or good Samaritans.***

After receiving a dead body from the police or relatives from home the following procedures should be followed

i. First get the information, document the police/transport ID/telephone number
ii. Prepare the trolley to receive the dead body
iii. Check the body appearance and attached properties, if it's a police case after documentations put the dead body directly to the cooling unit and wait for post-mortem exam to be done.
iv. Provide the mortuary identity card to the police, if will be later on provided to the relative
v. If it's a home cases after documentation inform the relatives about the other process of payments.
vi. After payments request the nearby relative to attend the embalming procedure then keep the dead body in the cooling unit after following the identification procedure and make sure it is well labelled.
vii. Make sure you label the form to indicate which unit the dead body has been put in.

### *9.1.15 Releasing the died body from mortuary to relatives*

Before giving out dead bodies following procedure should be followed;

i. For the dead body from the facility make sure the relative comes with the discharge summary, burial certificate and the proper receipt.
ii. Check if the information has been filled well in the mortuary register book.
iii. When relative have accomplished all the process and have all the form needed, assist them to prepare the dead body and keep it in a good condition

iv. Allow them to leave after finishing all the process.
v. For out - patients make sure the relatives come with the burial certificate/or letter from local government if they have it and proper receipts.
vi. When they have accomplished all process, assist them to prepare the body in the washer rooms and make sure equipment, water and sanitation is well controlled.

### *9.1.16 Handling personal property and clothing*

Personal property that arrives with decedent remains shall be processed as follows;

i. Decedent's personnel property will be entered into register book upon admission.
ii. Clothing should be described by colours and items
iii. Personal effect such as jewellery, watch as well as money should only be removed in the presence of relatives/police. **iv.** Personal effects such as jewellery should be described in non-valuable terms e.g. yellow metal or coloured stone**.**

### *9.1.17 Embalming procedure*

It consists of arterial infusion of embalming fluid (10% Formalin). Since most bodies are not disposed within 72hrs after death, hence the importance of a mortuary establishing this service.

i. Arrange all your equipment that are needed for embalming. ii. Prepare the formalin solution 10% concentration
iii. Put on the proper protective equipment
iv. Prepare the body to be embalmed
v. Start the embalming procedure
vi. Make sure that body has a label and tag
vii. Clean the area and the equipment that have been used.

### *9.1.18 Autopsy procedure*

i. Autopsy has to be complete routinely.
ii. A complete autopsy is defined to include a detailed external examination as entire body and an internal examination to include the removal and dissection of all thoraco –abdominal and neck organs, opening the head with the removal and examination of the brain
iii. A complete autopsy does not require histological examination
iv. A patient autopsy is defined as an examination that surface any part of the defined complete autopsy e.g. not opening any of the body cavities or not examining organs.
v. An external examination is defined as a detailed description of the decedents remains including scars, surgical incisions, medical devices and tattoos.

### *9.1.19 Pre-autopsy procedures*

Prior to autopsy the medical attendant will set up the autopsy work room according to the case examination status including preparing tables for body dissection, preparing instruments, preparing sample containers and collection tubes, preparing paper work for daily case load and taking radiographs.

Autopsy work room should be set up with the following instruments supplies, in certain cases it will be necessary to equip the autopsy work room with specialized instruments or additional supplies.

### *9.1.20 The standard autopsy precautions including using:*

- A surgical scrub or suits
- Surgical cap
- Impervious gown of apron with full sleeves coverage
- A form of eye protection e.g. goggles or face shield
- Shoe covers
- Double surgical gloves with interposed layer of cut proof synthetic mesh.
- Surgical masks that may protect nose and mouth from splashes of body fluids but do not always offer protection from airborne pathogens
- Use of respiration in adequate resources
- Safety practices to prevent injury from sharp items including hand washing as necessary after glove removal

### *9.1.21 Peri autopsy procedure*

i. Remove bodies from body storage colder in the stage in autopsy suits
ii. Identification by the relatives and authorised photographer can take photos
iii. Undress and transfer remaining to autopsy table
iv. Remove medical intervention devices and wash remains
v. Assist photographer in taking autopsy photographs and ID photos
vi. Perform initial Y or median incision
vii. Remove chest plate
viii. Open thoracic and abdominal cavities
ix. Medical attendant has to assist the pathologist in obtaining toxicology samples (blood, bile, urine, vitreous, gastric, liver, brain)
x. Remove organs
xi. Weigh and record organs weight
xii. Open the entire length of the gastro intestinal tract
xiii. Elevate head
xiv. Incise and reflect scalp
xv. Remove brain
xvi. Remove duct
xvii. Obtain decedents fingerprints
xviii. Perform other autopsy procedures as directed (removing spinal cord, opening inner extremities to exam pulmonary thrombo –emboli, stripping pavietal

pleural, incising the psoas muscles, assisting with preparation of sex kits and DNA cards. Measure the length of a died.

### *9.1.22 Post Autopsy Procedures*

i. Put organs in viscera bag ii. Replace body organs

iii. Close the thoracic, abdominal and cranial cavities with sutures
iv. Clean the body and replace in body bag
v. Indicate completion of examination by inciting a ONE on body bag
vi. Return body to refrigerated storage
vii. Put specimen in designated area depending on processing instructions;
    a) Toxicology samples are put in toxicology refrigerator in the yellow tray labelled" toxicology"
    b) Histology sections are put in the yellow tray on top of toxicology refrigerator labelled "histology"
    c) Microbiology sample are put in the yellow tray on top of toxicology refrigerator labelled "micro"
viii. Thoroughly clean and disinfect autopsy and dissection tables, sinks, drains, instruments, dry erase boards floor area
ix. Between examinations all instruments surfaces should be cleaned with a 10% bleach solution.

### *9.1.23 Body storage and organization*

i. Body should be stored in clean, closed body bags with no leakage of fluids on rack or tray
ii. Place body in empty compartment head toward the wall
iii. Tag body storage compartment with decedent name and case number
iv. Log body on body part(s) into box and cart inventory sheet
v. Maintain an accurate box or cart inventory sheet. The inventory sheet should be updated appropriate daily
vi. Empty trays to the cleaned and disinfected and kept in box
vii. All trays should be cleaned and disinfected following a release or transfer

### *9.1.24 Receiving and releasing remains*

**Receiving Remains;**

i. Identify ID bracelet with Police to tag
ii. Obtain appropriate signatures on transport notification form
iii. Take photography using the camera
iv. Obtain decedent height and weight
v. Record personal property (clothing)
vi. Complete intake of body into the logbook
vii. Complete intake of body in FACTS
viii. Log body on box and cart sheet ix. Place body in refrigerated storage

***Releasing Remains:***

i. Confirm in FACTS that the body is ready to be released and that the receipt of remain seen has been properly completed by communications unit.
ii. Print and sign copy of the Receipt of remains form.
iii. Sign body out of release logbook
iv. Obtain funeral home representatives signatures, number and initials where appropriate
v. Have to tag and receipt of remains form witnesses
vi. Clean and disinfect tray before retaining it to cold box

### *9.1.25 Sample Storage and Retrieval*

All stock specimens retained in clean and dry bottle in 10% buffered formalin label write the Post Mortem Examination Number, name, age, sex, address and anatomical site

### *9.1.26 Transportation of remains*

If a body is to be shipped out of the country, a letter stating that the autopsy showed no evidence of any infections or communicable diseases is required.

In the contents of such a letter must include Identification details such as name and date of death that match the details in the transit/burial permit or death certificate.

### *9.1.27 Supporting documents*

National guideline for operating of Mortuary services 2020, National guideline for establishment of Mortuary services 2020, IPC Guideline 2018

### *9.1.28 References*


1. Standard guidelines for the facilities and operation of mortuaries in Tanzania 2008
2. Hutchins GM. Practice guidelines for autopsy performance. Archives of pathology and laboratory medicine, 1994, 118(1):19-25
3. Advices ED, Sims KL. Enhancing autopsy performance and reporting. A system for a 5-day completion time. Archives of pathology and laboratory medicine,1996,120(3):249-53.

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## Annex 2: Biological Reference Intervals for Full Blood Count

| Parameter | Reference Range | | | | | |
|---|---|---|---|---|---|---|
| | Adult | | Infants | | Children | |
| | Males | Females | Birth | 1 month | 1 year | SI Unit |
| WBC | 4.00 - 10.0 | 4.0 - 10 | 10 - 26 | 5.0 - 9.0 | 6.0 - 16.0 | $x10^3$ /µL |
| NEU | 40 - 80 | 40 - 80 | 40 - 80 | 40 - 80 | 40 - 80 | % |
| LYM | 20 - 40 | 20 - 40 | 20 - 40 | 20 - 40 | 20 - 40 | % |
| MON | 2 - 10 | 2 - 10 | 2 - 10 | 2 - 10 | 2 - 10 | % |
| EOS | 1 – 6 | 1 - 6 | 1 - 6 | 1 - 6 | 1 - 6 | % |
| BASO | <1 - 2 | <1 - 2 | <1 - 2 | <1 - 2 | <1 - 2 | % |
| RBC | 4.5 - 5.5 | 3.8 - 4.8 | 5.0 - 7.0 | 3.0 - 5.4 | 3.9 - 5.1 | $x10^6$ /µL |
| HGB | 13.0 - 17.0 | 12.0 -15.0 | 14.0 - 22.0 | 11.5 - 16.5 | 11.1 - 14.1 | g/dL |
| MCV | 83.0 - 99.0 | 83.0 - 99.0 | 100 -120 | 92 - 116 | 72 - 84 | Fl |
| MCH | 27.0 - 32.0 | 27.0 - 32.0 | 31.0 - 37.0 | 30.0 - 36 | 25.0 - 29.0 | Pg |
| MCHC | 31.5 - 34.5 | 31.5 - 34.5 | 30.0 - 36.0 | 29.0 - 37.0 | 32.0 - 36.0 | g/dl |
| Hct | 40 – 50 | 36 - 46 | 45 - 75 | 33 - 53 | 30 - 38 | % |
| RDW | | | | | | |
| PLT | 150 - 410 | 150 - 410 | 100 - 450 | 200 - 500 | 200 - 550 | $x10^3$ /µL |
| MPV | | | | | | |

## Annex 3: Biological Reference Intervals for Coagulation Profile

| Test | Range | SI Unit |
|---|---|---|
| Prothrombin Time | 9.40 - 12.50 | Sec |
| Activated Prothrombin Time | 25.40 – 36.90 | Sec |
| Fibrinogen | 2.20 – 2.80 | g/l |
| Factor V | 0.62 -1.39 | IU |
| Factor VII | 0.50 -1.29 | IU |
| Factor VIII | 0.50 -1.50 | IU |
| Factor IX | 0.65 -1.50 | IU |
| Free protein S (male) | 74.10 - 145.10 | % |
| Free protein S (female) | 54.70 - 123.70 | % |
| Protein S activity | 63.50 - 149.00 | % |
| Protein C may be less in neonates, infants and increase in adolescence | 70.00 – 140.00 | % |
| Plaminogen (activity) | 80.20 - 132.50 | % |
| Plamin inhibitor | 98.00 – 122.00 | % |
| Homocysteine | 4.30 - 11.10 | µmol/L |
| D-dimmer | ≤ 232 | ng/ml |
| Von Will brand factor ristocetin cofactor activity (blood group O) | 480 - 201.90 | % |
| Von Will brand Factor ricostein factor activity (blood A+B+AB) | 60.80 - 239.80 | % |

## Annex 4: Biological Reference Intervals for Urine Biochemistry

| Parameter | Abbreviation | Biological Reference Intervals |
|---|---|---|
| Urobilinogen | URO | Normal |
| Glucose | GLU | Negative |
| Bilirubin | BIL | Negative |
| Ketones | KET | Negative |
| Specific gravity | S.G | 1.003-1.029 |
| Occult blood | BLD | Negative |
| Ph | Ph | 4.5 - 7.8 |
| Protein | PRO | Negative |
| Nitrite | NIT | Negative |
| Leukocytes | LEU | Negative |

## Annex 5: Biological Reference Intervals for Clinical Chemistry and Immunoassays

| Test Name | Normal range | SI Unit |
|---|---|---|
| **Parameter/Analyte** | **Sub category** | |
| **Alanine aminotransferase (ALT)** | 0 - 55 | U/L |
| **Albumin** | 35 - 50 | g/l |
| **Alkaline Phosphate** | Male 15 -125 | U/L |
| | Female 15 -125 | U/L |
| | Male child 0 - 500 | U/L |
| | Female Child 0 - 500 | U/L |
| | Children | U/L |
| | Aged 1 day <250 | U/L |
| | Aged 2 - 5 days <231 | U/L |
| | Aged 6 days - 6 months <449 | U/L |
| | Aged 7months - 1 year <462 | U/L |
| | Aged 1 - 3years <281 | U/L |
| | Aged 4 - 6years <269 | U/L |
| | Aged 7 - 12 years <300 | U/L |
| | Aged 13 - 17 years (M) <390 | U/L |
| | Aged 13 - 17 years (F) <187 | U/L |
| **Aspatate Aminotransferase** | 5 - 34 | U/L |
| **Bilirubin – Direct** | 0 – 8.6 | µmol/L |
| **Bilirubin – Total** | 3.4 - 20.5 General | µmol/l |
| **Total Protein** | 64 - 83 | g/l |
| **Gamma Glutamyl Transferase** | Male 12- 64 | U/L |
| | Female 9 - 36 | U/L |
| | Male child 9 - 36 | U/L |
| | Female child 9 - 36 | U/L |
| **CSF protein** | 0-4.3 lumbar fluid | g/L |
| **CSF glucose** | One third of Glucose | g/L |
| **Asciti Protein** | 60 - 80 | g/L |
| **Ascitic Glucose** | 70 - 100 | mg/dL |
| **Creatinine** | Adult Male 63.6 - 110.5 | µmol/L |
| | Adult Female 50.4 - 98.1 | µmol/L |
| | Male child 27 - 88 | µmol/L |
| | Female child 27 - 88 | µmol/L |
| **Blood Urea Nitrogen (BUN)** | Male 3.2 - 7.4 | µmol/L |
| | Female 2.5 - 6.7 | µmol/L |
| | Male child 3.2 - 7.4 | µmol/L |
| | Female Child 2.5 - 6.7 | µmol/L |
| **Cholesterol Total** | <5.2 | mmol/L |
| **HDL - Cholesterol** | 1.04 - 1.55 | mmol/L |
| **LDL – Cholesterol** | 0 - 3.34 | mmolL |
| **Triglycerides** | 0 - 1.69 | mmol/L |

| Test Name | Normal range | SI Unit |
|---|---|---|
| **Sodium ( Na )** | 136 - 145 | mmol/L |
| **Potassium ( K )** | 3.5 – 5.1 | mmol/L |
| **Chloride (Cl )** | 98 – 107 | mmol/L |
| **Amylase Total** | 25- 125 | U/L |
| **Creatine Kinase ( CK )** | Male 30 – 200 | U/L |
| | Female 29- 168 | U/L |
| | Male child 30 – 200 | U/L |
| | Female child 29-168 | U/L |
| **Lactate dehydrogenase (LDH)** | 125 – 220 | IU/L |
| **Lipase** | 13- 60 | U/L |
| **Calcium** | Male 2.1 – 2.55 | mmol/L |
| | Famale 2.1-2.55 | mmol/L |
| | Male child 2.2 – 2.7 | mmol/L |
| | Female child 2.2 – 2.7 | mmol/L |
| **Glucose** | 3.3 - 6.1 | mmol/L |
| **Iron** | Male 5.5 – 25.8 | µmol/L |
| | Female 4.5 - 25.8 | µmol/L |
| | Male child 5.5 – 25.8 | µmol/L |
| | Female child 4.5 – 25.8 | µmol/L |
| **% Saturation (Iron saturation)** | 20 - 50 | % |
| **Uric Acid** | Male 0.21 - 0.42 | mmol/L |
| | Female 0.15 - 0.35 | mmol/L |
| | Male child 0.21 - 0.42 | mmol/L |
| | Female child 0.15 - 0.35 | mmol/L |
| **Phosphorus** | 0.74 - 1.52 | mmol/L |
| **Sodium 24hrs Urine** | 27 – 287 | mmol/24 hours |
| **Potassium 24hrs Urine** | 25 – 125 | mmol/24hrs |
| **Transferrin** | Male 1.74 - 3.64 | g/L |
| | Female 1.8 - 3.82 | g/L |
| | Male child 1.86 – 3.88 | g/L |
| | Female child 1.86 - 3.88 | g/L |
| **Alpha Feto Protein** | 0.0 - 1.09 | ng/ml |
| **High Sensitive Troponin** | 13.8-17.5 Female | pg/ml |
| | 28.9-39.2 Male | pg/ml |
| **Vitamin B12** | 187-883 | pg/ml |
| **Ferritin** | 10 - 250 | ng/ml |
| **Folate** | 3.72 - 50.4 | ng/ml |
| **PSA** | 0.0 - 4.0 | ng/ml |
| **TSH** | 0.49 - 4.67 | IU/ml |
| **T4** | 0.47 - 4.67 | ng/L |
| **$T_3$** | 1.45 - 3.48 | pg/ml |
| **CK-MB** | 0.0 - 6 | % |

| Test Name | Normal range | SI Unit |
|---|---|---|
| **Tacrolimus** | 3 – 20 | ng/ml |
| **BNP** | 0-142 | pg/ml |
| **Cyclosporine** | 30.0-1500 | ng/ml |
| **CEA** | 0-5 | ng/ml |
| **CA-125** | 0-35 | IU/mL |
| **B-HCG** | Less than 5 for non pregnant | mIu/mL |
| | 25 for early pregnancy | mIu/mL |
| **Vitamin D** | 0 - 160 | ng/ml |
| **Immunoglobulin G** | 5.40-18-22 Male | g/l |
| | 5.52-16.31 Female | g/l |
| **Immunoglobulin E** | 1-12 months <15 | IU/mL |
| | 1-5 years <60 | IU/mL |
| | 6-9 years <90 | IU/mL |
| | 10-15 years <200 | IU/mL |
| | Adults <100 | IU/mL |
| **Immunoglobulin A** | Male 63 – 645 | mg/dl |
| | Female 65 – 517 | mg/dl |
| | Male child 21- 291 | mg/dl |
| | Female child 21 - 281 | mg/dl |
| **Immunoglobulin M** | Male 0.22-2.40 | g/l |
| | Female | g/l |
| | 0.33-2.93 | g/l |
| | Either 0.22-2.93 | g/l |
| **D - Dimer** | 0 .0 – 198 | ng/L |
| **CRP** | 0.0-5.0 | mg/L |
| **Estradiol** | Follicular phase 21-251 | pg/ML |
| | Midcycle phase 38-649 | pg/ML |
| | Lueal phase 21-312 | pg/ML |
| | Postmenopausal female 1028 | pg/ML |
| | Male 11-44 | pg/ML |
| **Prolactin** | Male | ng/ml |
| | 3.46-19.40 | ng/ml |
| | Female5.18-26.53 | ng/ml |
| **Testosterone** | Male 4.94-32.01 | nmol/L |
| | Female 0.38-1.97 | nmol/L |
| **Progesteron** | Follicular phase 0.1-0.3 | ng/ml |
| | Luteal phase 1.2-15.9 | ng/ml |
| | Postmenopausal 0.1-0.2 | ng/ml |
| | First trimester 2.8-147.3 | ng/ml |
| | Second trimester 22.5-95.3 | ng/ml |
| | Third trimester 27.9-242.5 | ng/ml |
| | Male 0.1-0.2 | ng/ml |
| **LH** | Male 1.14-8.75 | ng/ml |
| | Follicular phase 2.39-6.60 | ng/ml |
| | Midcycle peak-9.06-74.24 | ng/ml |

| Test Name | Normal range | SI Unit |
|---|---|---|
| | Luteal phase 0.909.33 | ng/ml |
| | Postmenopausal | ng/ml |
| | 10.39 - 64.57 | ng/ml |
| **FSH** | Male 0.95 - 11.95 | mIu/mL |
| | Follicular phase 3.03 - 8.08 | mIu/mL |
| | Midcycle peak 2.55 - 16.69 | mIu/mL |
| | Luteal phase 1.38 - 5.47 | mIu/mL |
| | Postmenopausal | mIu/mL |
| | 26.72 - 133.41 | mIu/mL |
| **Magnesium** | Male 0.66 - 1.07 | mmol/L |
| | Female 0.66 - 1.07 | mmol/L |
| | Male child 0.70 - 0.86 | mmol/L |
| | Female 0.70 - 0.86 | mmol/L |
| **ADA** | 0 - 15 | U/L |
| **Glycated haemoglobin (HBA1C)** | 4 - 6 | % |

## Annex 6: Critical or Panic Values that call for Immediate Actions

| **Analyte** | **Less Than** | **Greater Than** |
|---|---|---|
| Amylase | 25 U/L | 150 U/L |
| Chloride | 85 mmol/L | 115 mmol/L |
| CK | 30 U/L | 200 U/L |
| Creatinine | 26 umol/L | 120 umol/L |
| Glucose(fasting) | 2.5 mmol/L | 20.0 mmol/L |
| Potassium | 2.5 mmol/L | 6.0 mmol/L |
| Sodium | 120 mmol/L | 160 mmol/L |
| Bilirubin Total | 3.4 umol/L | 20.5 umol/L |
| Biliribun Total for new Born | **Newborn**<br>24hours ≥ 1374 umol/L<br>48hours ≥ 2224 umol/L<br>84hours ≥2904 umol/L<br>One week to one month ≥3424 umol/L | |
| Urea (BUN) | ≤1.0mmol/L | ≥ 54 mmol/L |
| HGB | < 5 mg/dl | > 20 g/dl |
| CD4 | 200 cells/□l | |

## Annex 7: Charts for Biochemical Identifications of Common Enterobacteriaceae and other Enteric Organisms

| Organism | MAC Reaction | TSI | Oxidase | $H_2S$ | Gas | Motility | Indole | Urea | Citrate | Haemolysis | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Serratia mercesens* | NLF | K/A or A/A | - | - | + | + | - | - | + | - | Red pigment at room temp on MHA |
| *Proteus mirabilis* | NLF | K/A | - | + | + | + | - | + | +(weak) | - | Grow with swarming xters on BA |
| *Proteus vulgaris* | NLF | A/A or K/A | - | + | + | + | + | + | +/- | - | Grow with swarming xters on BA |
| *Salmonella sp* | NLF | K/A | - | + | + | + | - | - | + | - | Citrate pos (non-typhoid salmonella) |
| *Salmonella typhi* | NLF | K/A | - | Wk+ | + | + | - | - | - | - | Black ppt on SSA&XLD |
| *Shigella sonnei* | NLF | K/A | - | - | - | - | - | - | - | - | No black ppt on SSA/XLD |
| *Other Shigella sp* | NLF | K/A | | - | - | - | - | - | - | - | |
| *Vibrio cholerae* | NLF | A/A | - | + | + | - | - | - | - | + | String test-positive |
| *Vibrio parahaemolyticus* | NLF | K/A | + | - | - | + | + | - | + | + | |
| *P.aeruginosa* | NLF | K/NC | + | - | - | + | - | + | + | +/- | Green pigmentation on MHA |
| *Acinetobacter spp* | NLF | NC | - | - | - | - | - | - | - | - | Coccoide rods |
| *Morganella morganii* | NLF | K/A | - | - | + | + | + | + | - | - | |
| *Providencia spp* | NLF | K/A | - | - | | + | + | | + | - | |
| *Yersinia enterocolitica* | NLF | K/A | - | - | - | +(25/-35°C) | +/- | +/- | - | - | |
| *Edwardsiella tarda* | NLF | K/A | - | + | + | + | + | - | - | - | |
| *E.coli* | LF | A/A | - | - | + | + | + | - | - | +/- | Grows with precipitate bile salt on MCA |

| Organism | MAC Reaction | TSI | Oxidase | $H_2S$ | Gas | Motility | Indole | Urea | Citrate | Haemolysis | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Enterobacter aerogenes* | LF | A/A | - | - | + | + | - | - | + | - | Often resistant to Ampicillin and cephalosporin |
| *Klebsiella pneumoniae* | LF | A/A | - | - | + | - | - | + | + | - | Grow with very mucoid colonies |
| *Klebsiella oxytoca* | LF | A/A | - | - | + | - | + | + | + | - | |
| *Citrobacter freundii* | Late LF | A/A or K/A | - | + | + | + | - | +/- | + | - | |

# NOTE PAD

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