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TANZANIA**



MINISTRY OF HEALTH

**National Medical Laboratory
Standard Operating
Procedures for Dispensaries**

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NATIONAL MEDICAL STANDARD OPERATING PROCEDURES FOR DISPENSARY 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
BMC	Bugando Medical Centre
BRM	Bio risk Management
BSC	Biological Safety Cabinet
BSc	Bachelor of Science
BSL	Biosafety Level
BUQ	Bottom-Up Quantification
CCM	Chama Cha Mapinduzi (<i>Ruling Party in the United Republic of Tanzania</i>)
CD4	Cluster of Differentiation 4
CDC	Centres for Disease Control and Prevention
CDs	Cluster of Differentiations
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
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
DLTs	District Laboratory Technologists
DMO	District Medical Officer
DO	Data Officer
DP	Development Partner
DQA	Data Quality Assurance

DTS	Dried Tube Sample
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
IATA	International Air Transport Association
ICAP	International Centre for AIDS Care and Treatment Programs
IDSR	Integrated Disease Surveillance and Response
IHR	International Health Regulations
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
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	Non-Communicable Diseases
NGO	Non-Governmental Organization
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
NSOPML	National Standard Operating Procedures for Medical Laboratories
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
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 organizations 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 organization. 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 organization'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.



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PERMANENT SECRETARY

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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 process of developing this document.



Prof. Tumaini J. Nagu
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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 instructions 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 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 ONE: 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 (sputum or urine), so he/she should be instructed properly and accordingly.
- v. Sufficient quantity of samples is to be collected to permit complete examination.
- vi. Samples are to be placed in sterile containers.
- vii. Some samples are directly collected in culture media. Contact laboratory if such collection is required.
- viii. Proper labelling of samples should always be done with patient's name, test type, date and site of collection etc.
- ix. The relevant clinical information is to be recorded on the request form.
- x. Any condition, circumstances or situation that will require special procedures should also be noted on the request form.
- xi. 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.
- xii. The most appropriate samples for isolation of viral, chlamydial or rickettsial agents depend on the nature of the illness.

- xiii. 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.
- xiv. 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.
- xv. 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.

2.0 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).

1.1.5 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.

1.1.6 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.

1.1.7 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.
- iv. Check the request form for the requested investigations and Inform the patient about the samples to be collected.
- v. Where possible, ask the patient to remove any tight - fitting sleeved clothing, or to roll up loose sleeves.
- vi. Label the containers before obtaining sample.
- vii. 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.
- viii. 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.
- ix. 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)
- c. in everwidening circles until the entire
- d. chosen area has been saturated with iodine.
- e. Allow the iodine to dry on the skin for at least 1 min.



- f. Completely remove the iodine with 70% alcohol/spirit swab following the pattern of application.
- x. 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.
- xi. With bevel up, insert the correct needle size on the veins at an angle between 15 - 30° then draw the blood into the appropriate collection tubes, (make sure the patient arm in a downward position to prevent reflux).
- xii. 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.
- xiii. Release the tourniquet 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.
- xiv. Apply pressure with thumb on antiseptic swab at puncture site for 2 - 4 min till the blood ooze stops.
- xv. If syringe was used, safely remove the needle from the syringe before distribution.
- xvi. 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;

- First tube blood culture,
- Second tube: non-additive tube (e.g. red stopper),
- Third tube: coagulation tube (e.g. blue stopper),
- Last tube: additive tube (e.g. lavender or green tube).

1.1.8 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.

1.1.9 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.
- v. Clean the heel site (i.e. gauze and water) if the foot appears unclean (e.g. faecal material).
- vi. Encircle the foot with the palm of the hand and the index finger.
- vii. Make a quick puncture with the automated lancet device
- viii. Wipe off the first drop of blood with a gauze swab
- ix. Collect the sample with the second drop of blood using any of the collection devices such as slides or rapid test
- x. Apply pressure with a clean dry gauze pad until bleeding stop

3.0 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 alkalize 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.

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.

1.1.10 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 mid-stream 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.
- 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. 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.

1.2 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.

1.2.1 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.

4.0 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.

1.2.2 Cerebrospinal fluid (CSF)

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

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.

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.

1.2.3 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.

1.2.4 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 intra-abdominal malignant disorders.

1.2.5 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

1.2.6 Hydrocele Fluid

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

Collection of aspiration fluids (effusions)

- Collection of synovial, pleural, pericardial, peritoneal, or hydrocele fluid is carried out by a medical officer or competent nurse.
- Label each container with the date and the patient's identifiers
- 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).
- If the sample cannot be examined immediately, fluid should be frozen and stored at -70°C until examined.

5.0 COLLECTION OF GENITAL SAMPLES

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

1.2.7 Urethral swabs

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

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.

- v. When culture is indicated (see previous test),
Collect a sample of pus on a sterile cotton-wool swab.
If possible, before inserting the swab in a container of Amies transport medium, inoculate a plate of culture medium.
- vi. Label the samples and deliver them to the laboratory as soon as possible.
- vii. 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).

Cervical swabs and possible pathogens

From non-puerperal women:

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

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*.

1.2.8 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.

1.2.9 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.

1.2.10 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.

1.2.11 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 dark-field microscopy

6.0 WOUND SAMPLES

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 Gram-negative rods, *Streptococcus pyogenes*, *Clostridium* species and other anaerobic bacteria

Collection of Wound samplless - *General considerations*

Samples should be collected by a medical officer or an experienced nurse.

Pus from an abscess is best collected at the time the abscess is incised and drained, or after it has ruptured naturally.

When collecting pus from abscesses, wounds, or other sites, avoid contaminating the sample with commensal organisms from the skin.

As far as possible, a samples from a wound should be collected before an antiseptic dressing is applied.

When pus is not being discharged, use a sterile cotton-wool swab to collect a sample from the infected site.

Immediately after collection, immerse the swab in Amies transport container.

Label the samples and as soon as possible deliver it with a completed request form to the laboratory.

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.

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

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.

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

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

- i. Using a sterile technique, aspirate or collect from a drainage tube up to 5 ml of pus.
- ii. 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.

7.0 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.

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.

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:

6.1 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.

6.2 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

6.3 Collection of Nasopharyngeal Swabs

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

- i. Label 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 nasopharynx 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

7 COLLECTION OF OROPHARYNGEAL SWABS

Assemble equipment for oropharyngeal 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

8 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):

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

Sputum Collection Procedure

1. Label the sample container on the body of the container, not on the lid and fill out the sputum examination request form.
2. 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
3. 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.
 4. 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.
 5. Encourage the patient to bring the collected sample back to the unit as quickly as possible.
 6. 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.
 7. *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.
 8. 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)

5.1. 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

5.2. Scope

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

5.3. Responsibility

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

5.4. 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 (pLDH) 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.

5.5. Sample Requirements

Whole blood from EDTA/Finger prick

5.6. Equipment

Timer

5.7. Materials

Gloves, Test kit, Lab Coat, and

5.8. Storage and Stability

MRDT sample should be tested within 1hour after collection if not possible stored at 2-8⁰c for 7 days.

5.9. 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) f

5.10. Calibration

Not Applicable

5.11. Quality Control

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

5.12. 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).

5.13. Biological Reference Intervals

Not Applicable

5.14. Interpretation and Reporting of Results

Negative

The presence of one colour band (“C” Control line) within the result window indicates a negative result.

Positive

“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

“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

If no coloured band appear, at control line “c” within stipulated time then the result is invalid.

5.15. 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.

5.16. Performance Characteristics

Refer to package insert

5.17. Supporting Documents

Sample collection manual

5.18. 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 Responsibility

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

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

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.

2.2.7 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.

2.2.8 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.

2.2.9 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

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 2 μ l of blood and pour it on the smallest circle on the slide card
- vi. Place the spreader in front of the 2 μ l drop of blood at a 30°- 45° 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 6 μ l 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

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°C and shake at interval.
- iv. Filter the stock solution before storing in a cool dry place with label and date of preparation

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: 3mls of Giemsa +25ml of buffered water PH of 7.2
- iii. Mix and transfer to a clean capped leakproof bottle
- iv. Lable and keep in a dry place

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

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 x 100 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

2.2.13 Biological Reference Intervals

Not Applicable

2.2.14 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

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

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”

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 <i>P.falciparum</i> Asexual/200wbc's
Malaria for > 5 years& adult	>1000 <i>P.falciparum</i> Asexual/200wbc's
None Tropical people	ANY POSITIVE

2.2.15 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

2.2.16 Performance characteristics

Refer to the method verification report of this procedure.

2.2.17 Supporting Documents

Not Applicable

2.2.18 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 Responsibility

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

2.3.4 Principle

Macroscopic Examination

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

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 1 hour of collection, if not possible refrigerate at 2-8°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 infectious.
- 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 planned

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

Macroscopic examination

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

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.
- v. Centrifuge the urines at 3000rpm for 5 minutes.
- vi. Remove the centrifuge tubes from the centrifuge
- vii. Pour off the supernatant into the sink with running water and leave the urine deposit
- viii. Transfer the rack containing arranged centrifuge test tubes to the working area
- ix. Arrange the marked slides according to the number of urine sample
- x. Re-suspend the urine deposit by tapping the bottom of the centrifuge tube.
- xi. Transfer a drop of the deposit onto a clean glass slide.
- xii. Put on a cover slip
- xiii. Place the slide on the microscope stage and reduce the iris diaphragm
- xiv. 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).
- xv. Swing the x40 objective into position
- xvi. 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).
- xvii. structures cannot be seen if a very bright light is used
- xviii. 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

Macroscopic

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

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.”

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 abnormalities/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.

2.4.3 Responsibility

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

2.4.4 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.

2.4.5 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.

2.4.6 Equipment

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

Maintenance

Maintenance of microscopes should be done as planned

2.4.7 Materials

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

2.4.8 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.

2.4.9 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.

2.4.10 Calibration

Calibration of centrifuge should be done as per schedule

2.4.11 Quality Control

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

2.4.12 Procedure

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.

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
- v. For mucoid or watery stool, mix the entire contents before picking a portion to mix with saline.
- vi. Cover the preparations with a cover slip.
- vii. Start with 10x objective to systematically, examine the entire saline preparation.
- viii. 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.'
- ix. Use the iodine preparation to assist in the identification of cysts.
- x. Report the findings on Stool analysis worksheet.
- xi. Discard the used microscope slide on the container with disinfectant.

2.4.13 Biological Reference Intervals

Not Applicable

2.4.14 Interpretation and Reporting of Results

Interpretation

- i. Adult helminths or portions of helminths may be recovered and seen with a naked eye. Examples include *E. vermicularis* 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 different 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 samples 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 associated 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*

Reporting

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*.

Microscopic Findings – Report the Following

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

2.4.15 Limitation of the Procedure and Sources of Error

Delay in examination of stool sample may cause missing of some parasites in wet preparation which are detected still alive. Examples of such organisms are *Strongyloides stercoralis*, *Giardia lamblia* *E. Histolytica* trophozoites, etc.

Presence of urine kills trophozoites (false-negative results).

Excessive heat or cold.

2.4.16 Performance Characteristics

Refer to the method verification of this procedure.

2.4.17 Supporting Documents

Not Applicable

2.4.18 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.

CHAPTER THREE: BLOOD TRANSFUSION

3.0 PROCEDURE FOR ABO AND RHESUS BLOOD GROUPING

3.0.1 Purpose

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

3.0.2 Scope

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

3.0.3 Responsibility

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

3.0.4 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).

3.0.5 Sample Requirements

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

3.0.6 Equipment

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

3.0.7 Materials

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

3.0.8 Storage and Stability

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

3.0.9 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.

3.0.10 Calibration

Calibration of auxiliary equipment should be done as per calibration schedule

3.0.11 Quality Controls

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

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.

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.

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

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.

Interpretation of IQC results

Commer cial Antisera	Cell suspensions						
	A cells	B cells	AB cells	Rh D pos cells	Rh D Neg Cells	O sensitized cells	Un sensitised 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)

3.0.12 Procedure Steps

Prepare of 2- 5% cell suspension

- Place 2-3 drops of donor red blood cells into a tube
- Fill the tube (3/4) with 0.85% normal saline
- Centrifuge the tube at 3400rpm for 2 to 3 minutes. Decant supernatant fluid. (Repeat 3 times)
- 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

Blood grouping procedure

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.

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,
- iv. Add one drop of B reagent cells into tube labelled B cells
- v. Mix the contents of the tubes gently
- vi. Centrifuge the tubes at 1000 rpm for one minute.
- vii. Examine the serum for evidence of haemolysis, gently suspend the cell bottoms and examine them for agglutination macroscopically and microscopically.
- viii. Read, interpret and record test results.

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:
- viii. Wash contents of the tube 3 times with physiological saline
- ix. Discard supernatant after third wash
- x. Add one drop Anti-Human Globulin Serum (AGS)
- xi. Spin for 1000rpm for one minute
- xii. Observe for agglutination (macroscopically and microscopically) or haemolysis
- xiii. If agglutination is observed at this stage, report results as Rh (Du) Positive
- xiv. If reaction is still negative, add one drop of O sensitised cells to the tube
- xv. Spin for 1000rpm for 1minute
- xvi. Observe for agglutination (macroscopically and microscopically) or haemolysis
- xvii. Presence of agglutination or haemolysis indicates a valid negative result.
- xviii. 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.

3.0.13 Biological Reference Intervals

Not Applicable

3.0.14 Interpretation and Reporting of Results

Interpretation of results

Patient/Donor Cell Grouping					Patient/ Grouping	Donor	Serum
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

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

3.0.15 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 allo-antibodies that can interfere with antigen – antibody reactions

3.0.16 Performance Characteristics

Refer to the method verification report

3.0.17 Supporting Documents

Sample collection manual, safety manual, and quality manual.

3.0.18 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)
- iv. 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.

3.1 PROCEDURE FOR ESTIMATION OF HEMOGLOBIN BY USING COPPER SULPHATE SOLUTION

3.1.1 Purpose

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

3.1.2 Scope

This procedure provides guidance on haemoglobin estimation of blood donors using copper sulphate (CUS04) method in NBTS Blood collection teams and its satellites.

3.1.3 Responsibility

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

3.1.4 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.

3.1.5 Sample Requirements

Capillary Blood

3.1.6 Equipment

Not applicable

3.1.7 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

3.1.8 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
- vi. *NB: Calculate expiry date six months from date of preparation*
- vii. Store and keep stock solution at room temperature in a tightly capped brown glass bottle.

3.1.9 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

3.1.10 Storage and Stability

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

3.1.11 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 infectious.
- 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.

3.1.12 Calibration

Not applicable

3.1.13 Quality Control

Reagent : Test specific gravity of copper sulphate solution using Hydrometer

Accept the copper sulphate solution if the specific gravity is 1.053

Reject copper sulphate if specific gravity is not equal to 1.053

Record findings of the copper sulphate inspection and testing report form.

Then select sample with slightly below and slightly high with a cut off value of High (12.6 -13.0) g/dl and Low (12.0 – 12.4) g/dl.

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

3.1.14 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 Hemoglobin
- 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 uninterrupted 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.

3.1.15 Biological Reference Intervals

Above or equal 12.5 g/dl

3.1.16 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

3.1.17 Limitations of The Procedure and Sources of Error

Not applicable

3.1.18 Performance Characteristics

Not applicable

3.1.19 Supporting Documents

Sample collection manual, Quality manual, Safety manual

3.1.20 References

AAB technical manual 12th edition, AfSBTC 4th Edition.

3.2 PROCEDURE FOR COMPATIBILITY TESTING

3.2.1 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)

3.2.2 Scope

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

3.2.3 Responsibility

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

3.2.4 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.

3.2.5 Sample Requirements

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

3.2.6 Equipment

Centrifuge, Water bath, timer, refrigerator

3.2.7 Materials

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

3.2.8 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

3.2.9 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 infectious.
- iv. Refer to National infection prevention and control Guidelines for health waste management and safety practice.

3.2.10 Calibration

Calibration should be done as per schedule.

3.2.11 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.

3.2.12 Procedure Steps

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 donor cells 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

Interpretation and reporting:

Agglutination or hemolysis 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 Anti-globulin test. If there is any incompatibility, immediately call the ward to stop the transfusion and re-call the blood unit.

3.2.13 Biological References Intervals

Not applicable

3.2.14 Reporting and Interpretation of results

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.

Reporting of Results

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

3.2.15 Limitation of the Procedure and Sources of Errors

Hemolysis 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

3.2.16 Performance Characteristics

Refer to the method verification report.

3.2.17 Supporting Documents

Quality manual, sample collection manual and safety manual

3.2.18 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 URIT-12 HEMOGLOBIN METER

4.1.1 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.

4.1.2 Scope

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

4.1.3 Responsibility

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.

4.1.4 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.

4.1.5 Sample Requirements

Fresh capillary or EDTA-anticoagulated venous whole blood

4.1.6 Equipment

URIT-12 Hemoglobin Meter

4.1.7 Materials

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

4.1.8 Storage and Stability

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

4.1.9 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 guideline

4.1.10 Calibration

Not Applicable

4.1.11 Quality Control

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

4.1.12 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 13-15 μ l 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.

4.1.13 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

4.1.14 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 \leq (5.07) g/dl is considered as critical and communicate with clinician

4.1.15 Limitation of the Procedure and Sources of Errors

Only whole blood or EDTA anticoagulated blood should be used.

4.1.16 Performance Characteristics

Refer into method verification report

4.1.17 References

URIT – 12 Hemoglobin meter Operation Manual

4.2 PROCEDURE FOR DETERMINATION OF HAEMOGLOBIN LEVEL USING HEMOCHROMAX PLUS

4.2.1 Purpose

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

4.2.2 Scope

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

4.2.3 Responsibility

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.

4.2.4 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.

4.2.5 Sample Requirements

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

4.2.6 Equipment

Hemochroma PLUS machine

4.2.7 Materials

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

4.2.8 Storage and Stability

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

4.2.9 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.

4.2.10 Calibration

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

4.2.11 Quality Control

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

4.2.12 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. Press 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

4.2.13 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

4.2.14 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 \leq (5.07) g/dl is considered as critical and communicate with clinician

4.2.15 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

4.2.16 Performance Characteristics

Refer to method verification report

4.2.17 Supporting Documents

Sample Collection Manual, Safety Manual,

4.2.18 References

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

- Hemochromax PLUS user manual

4.3 PROCEDURE FOR DETREMANATION OF HEMOGLOBIN LEVEL USING HEMOCUE 201+ MACHINE

4.3.1 Purpose

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

4.3.2 Scope

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

4.3.3 Responsibility

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

4.3.4 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

4.3.5 Sample Requirements

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

4.3.6 Equipment

Hemocue 201+ machine

4.3.7 Materials

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

4.3.8 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.

4.3.9 Safety

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

4.3.10 Calibration

Not Applicable

4.3.11 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.

4.3.12 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. Press 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.

4.3.13 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

4.3.14 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 \leq (5.07) g/dl is considered as critical and communicate with clinician

4.3.15 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

4.3.16 Performance Characteristics

Refers into method verification report

4.3.17 Supporting Documents

Waste Management Procedure /IPC guideline

Quality Control Result Procedure

4.3.18 References:

Monica Cheesbrough. District laboratory practice in tropical countries, Part 2. 2000.
Hemocue Hb 201+ cuvette kits insert and use manual.

4.4 PROCEDURE FOR CD 4 COUNT TEST BY USING BD FACS PRESTO

4.4.1 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.

4.4.2 Scope

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

4.4.3 Responsibility

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.

4.4.4 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.

4.4.5 Sample Requirements

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

4.4.6 Equipment

BD FACS Presto

4.4.7 Materials

Reagent	Consumables
BD FACS presto cartridge	Blue and yellow tips BD FACS presto print out paper BD disposable 100 µl Pipette

4.4.8 Storage and Stability

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

4.4.9 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.

4.4.10 Calibration

Not Applicable.

4.4.11 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

4.4.12 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.

4.4.13 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

4.4.14 Interpretation and Reporting of Results

Interpretation of results

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

Reporting of Results

- Report the obtained results as displayed on the screen.

Critical values

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

4.4.15 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

4.4.16 Performance Characteristics

Refer to Method verification report

4.4.17 Supporting Documents

Quality Manual, Sample Collection Manual, Safety Manual.

4.4.18 References

Becton Dickinson BD FACS presto Operating Manual

4.5 PROCEDURE FOR FULL BLOOD COUNT BY USING URIT BH – 40P HAEMATOLOGY ANALYSER.

4.5.1 Purpose

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

4.5.2 Scope

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

4.5.3 Responsibility

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.

4.5.4 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).

4.5.5 Sample Requirements

Whole blood sample in EDTA-K₂H₂O tube.

4.5.6 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

4.5.7 Materials

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

4.5.8 Storage and Stability

- Keep the Set of Controls at 2°C – 8°C
- Never use reagent and control beyond its expiration date

- Blood sample be kept at temperature between 2°C- 8°C for 7 days.

4.5.9 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.

4.5.10 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

4.5.11 Quality Control

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

4.5.12 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.

4.5.13 Biological Reference Interval

See annex 1.

4.5.14 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 x10⁹/L, HGB <5g/dL, PLT < 50 or 1000 x10⁹/L

4.5.15 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.

4.5.16 Performance Characteristics

Refer to the method verification report of this procedure.

4.5.17 Supporting Document

Sample collection manual

4.5.18 References

URIT BH- 40P Operation manual

4.6 PROCEDURE FOR FULL BLOOD COUNT USING OF ABX PENTRA 80 HAEMATOLOGY ANALYSER.

4.6.1 Purpose

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

4.6.2 Scope

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

4.6.3 Responsibility

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.

4.6.4 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.

4.6.5 Sample Requirements

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

4.6.6 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.

4.6.7 Materials

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

4.6.8 Storage and Stability

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

4.6.9 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.

4.6.10 Calibration

Calibrate the ABX Pentra 80 analyser under the following conditions:

- i. Change of software
- ii. Major component replacement

4.6.11 Quality Control

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

4.6.12 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.

4.6.13 Biological Reference Interval

See annex 1.

4.6.14 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

4.6.15 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 °C 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 and 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, cryoglobulins and red cell fragments.

4.6.16 Performance Characteristics

Refer to the method verification report of this procedure.

4.6.17 Supporting Document

Sample collection manual

4.6.18 References

ABX PENTRA 80 operator's manual

4.7 PROCEDURE FOR FULL BLOOD COUNT USING SINNOWA HB - 7021

4.7.1 Purpose

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

4.7.2 Scope

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

4.7.3 Responsibility

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.

4.7.4 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.

4.7.5 Sample Requirements

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

4.7.6 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

4.7.7 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

4.7.8 Storage and Stability

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

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

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

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

- Change of software
- Major component replacement

4.7.11 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:-
 - Before analyzing the samples
 - After replacement of the reagents
 - 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

4.7.12 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. Press the Touch Plate to activate aspiration
- x. Remove the tube when the beep sounds and replace the cap.
- xi. When the cycle is finished, the results post to the Data log and are displayed in the Run View
- xii. Print the results

4.7.13 Biological Reference Interval

See annex 1.

4.7.14 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

4.7.15 Limitation of the Procedure and Sources of Error

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

4.7.16 Performance Characteristics

Refer to the method verification report of this procedure.

4.7.17 Supporting Document

Sample collection manual

4.7.18 References

- SINNOWA HB – 7021 Hematology Analyser user manual.

4.8 PROCEDURE FOR FULL BLOOD COUNT USING BHA 3000 HAEMATOLOGY ANALYSER

4.8.1 Purpose

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

4.8.2 Scope

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

4.8.3 Responsibility

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.

4.8.4 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

4.8.5 Sample Requirements

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

4.8.6 Equipment

BHA 3000 Haematology Analyzer, Refrigerator

4.8.7 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

4.8.8 Storage and Stability

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

4.8.9 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.

4.8.10 Calibration

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

- i. Change of software
- ii. Major component replacement

4.8.11 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

4.8.12 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. Press the press Plate to activate aspiration
- x. Remove the tube when the beep sounds and replace the cap.
- xi. When the cycle is finished, the results post to the Data log and are displayed in the Run View
- xii. Print the results

4.8.13 Biological Reference Interval

See annex 1.

4.8.14 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

4.8.15 Limitation of the Procedure and Sources of Error

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

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

BHA 3000 Haematology Analyser user manual.

District Level

4.9 PROCEDURE FOR PERFORMING FULL BLOOD COUNT BY USING MS4 HAEMATOLOGY ANALYSER

4.9.1 Purpose

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

4.9.2 Scope

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

4.9.3 Responsibility

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.

4.9.4 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.

4.9.5 Sample Requirements

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

4.9.6 Equipment

MS4-S Hematology Analyser, and roller mixer

4.9.7 Materials

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

4.9.8 Storage and Stability

Blood is stable for about 4 hours at room temperature or 24hours at 2- 6oC

4.9.9 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

4.9.10 Calibration

All auxiliary equipment should be calibrated annually

4.9.11 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.

4.9.12 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.

4.9.13 Biological Reference Intervals

See annex 1.

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

4.9.14 Interpretation and Reporting of Results

Results are reported and communicated through the appropriate locally established procedure

4.9.15 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.

4.9.16 Performance Characteristics

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

4.9.17 Supporting Documents

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

4.9.18 References

MS4S operator's manual, 2013-07

CHAPTER FIVE: CLINICAL CHEMISTRY AND IMMUNOLOGY

5.1 PROCEDURE FOR BLOOD GLUCOSE BY USING ACCUCHECK GLUCOMETER

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 management 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 Responsibility

Qualified and competent registered and licensed Health laboratory practitioners 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 dehydrogenase). 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 proportional 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 examining 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 diabetes.
- 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 needed in the interpretation of neoNot applicable 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 Performance Characteristics

Refer to the verification report

5.1.17 Supporting Document

Not applicable

5.1.18 References

ACCU-CHEK Active 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 Responsibility

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 diabetes.

- 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 Performance 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 Responsibility

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 annual

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 Responsibility

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°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 request 10-20 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°C – 8°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 PROCEDURE 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 Responsibility

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

Perform the **CYBOW™ 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 infectious.
- Front cover of machine should be covered during operation to avoid sample contamination.
- Used only power cord specified for CYBOW™ 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 examining 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 one 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 placed on strip loading plate, press **Enter** button.
- After incubation time of the 1st strip, it starts to read and print result of the strip one by one.

3. Quick mode

- Select the quick mode by pressing 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/Standard, 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 guideline
- ii. All personal protective equipment (PPE) must be worn when performing this procedure.
- iii. All samples must be regarded as potentially infectious.

5.6.10 Calibration

- i. Perform 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 should 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

Performed 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

Hemolyzed, 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 Responsibility

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 Chemistry 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 semi-automated 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 Responsibility

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°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°C 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°C 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 Clindiaq FA 200 analyzer.

5.9.2 Scope

This procedure is used at Clinical chemistry section for processing basic chemistry tests using Clindiaq FA 200 analyzer.

5.9.3 Responsibility

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 states that the absorptive capacity of a dissolved substance is directly proportional to its concentration in the solution.

5.9.5 Sample Requirements

Serum is the most preferred sample.

Refer to the facility Laboratory sample collection manual.

5.9.6 Equipment

Clindiaq 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°C after testing for 3 days.

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 infectious.
- 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 perform 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 process 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 Clindiaq 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 Clindiaq 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 Responsibility

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 unprocessed 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 performed 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 frozen samples can not be used for testing due to loss of enzyme or hormone activities

5.10.16 Performance Characteristics

Refer 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 Responsibility

Qualified and competent registered Health Laboratory practitioners 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 antigen in the sample, the gold-labeled antibody in the colloidal gold pad or nitrocellulose membrane, and the antibody pre-coated on the test line can form a purplish red streak on the test line. The colour intensity of the test line is proportionate to the quantity of antigens detected in the sample. The analysis system can obtain the photo-electric signal intensity of the complex by 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 voltage difference has a linear relationship with the antigen concentration which can 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 2-8°C not more than 3 days.

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 infectious.
- 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 biohazard waste before disposal.

5.11.10 Calibration

Perform 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
Bilirubin Total for new Born	Newborn 24hours ≥ 1374umol/L 48hours ≥ 2224umol/L 84hours ≥2904umol/L 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 Responsibility

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°C in sealed foil pouches and only stable until expiration date. If not refrigerated they can be stored at room temperature(15-25°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° 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 guideline
- 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		<p>Touch  to enter the patient sample mode.</p> <p>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.</p> <p><i>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.</i></p>
2		<p>Insert the Test Cartridge with the barcode label facing left.</p> <p><i>Be sure that the Test Cartridge is correctly placed in the cartridge chamber.</i></p>
3		<p>Close the lid manually. The Analyzer will start processing the Test Cartridge.</p> <p><i>The processing time depends on the test in use.</i></p>
4		<p>Touch  and enter the patient ID.</p> <p>Touch  to confirm.</p> <p>Touch  and enter the control ID or Alere Afinion™ Control Data.</p> <p>Touch  to confirm.</p> <p><i>Entering the patient ID, control ID or Alere Afinion™ Control Data will not interrupt the processing.</i></p>
5		<p>Record the result, then touch  to accept.</p> <p>If a printer is connected, touch  to print the result.</p> <p>The lid opens automatically.</p> <p><i>The result will be saved in the result records.</i></p>
6		<p>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.</p> <p><i>Keep the lid closed to protect the cartridge chamber when the Analyzer is not in use.</i></p>

5.12.13 Biological References

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 Responsibility

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 Known Positive control, Known Negative control	Marker pen Examination Gloves

6.1.8 Storage and Stability

- The kit should be stored at 2-30 °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°C.
- Store Serum and plasma sample at 2-8°C for up to 3 days.
- For long term storage, serum/plasma should be kept below -20°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 HIV-1/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 Responsibility

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°C and 30°C or as per manufacturer claims

Whole blood; If the blood sample is not immediately tested, it should be refrigerated at 2-8°C for 3days

Plasma or serum; If plasma or serum sample is not tested immediately, it should be refrigerated at 2-8°C for 7 days

For storage period longer than 2week, freezing below -20°C is required. They should be brought to room temperature 15-30°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 Biotline TM 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-1 and 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 Responsibility

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™ HIV test-kit, Disposable gloves, Laboratory coat, 70% alcohol

6.3.8 Storage and stability

Uni-Gold™ HIV test device and wash solution should be stored between 2-27°C or as per manufacturer instructions

Whole blood specimen should be stored at 2-8°C for up to 3 days at -20°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

- Bring reagents and samples to room temperature at least 20 minutes before use.
- Remove the test device from its protective wrapper.
- Label the device with sample identification number or patient/client identification number.
- Peel the foil cover from the reagent area of the test strips.
- For serum or plasma or whole blood collected by finger prick or venipuncture samples;
- Using one of the disposable pipettes supplied with the kit, fill it with the sample.
- Holding the pipette over the sample port, add two drops of sample (approximately 60 µl) carefully to the sample port of the test device.
- Add two drops (approximately 60 µl) of wash reagent to sample port and start the timer.
- 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 whether 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

- Avoid hemolyzed sample and beware of lipemic samples when interpreting results.
- 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.
- Intensity of the patient bar does not necessarily correlate to the titer of antibody in the sample.
- 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 Responsibility

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 Gonadotropin One Step Pregnancy Test Strip (urine) is rapid chromatographic immunoassay for the qualitative detection of Human Chorionic Gonadotropin in urine to aid in early detection of pregnancy. The test uses two lines to indicate results. The test line is pre coated with a monoclonal Human Chorionic Gonadotropin antibody to selectively detect elevated level of Human Chorionic Gonadotropin. The control line is pre coated with goat anti-mouse IgG antibody. The test also includes a burgundy coloured conjugate pad containing another monoclonal 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, evening 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° C up to expiration date or as per manufacturer instruction

If sample cannot be tested within 1 hour of collection, it should be stored at 2 - 8°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 infectious.

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

6.4.19

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 Responsibility

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, Laboratory coat Micropipette

6.5.8 Storage and stability

- a. The test device in the sealed pouch can be stored at 2-40⁰C 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 2-8⁰c for up 3 days
- c. Serum and plasma maybe stored at 2-8⁰c for up 7 days, for long term storage, serum and plasma specimens should be kept at-20⁰c 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 (approximately 30ul) 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 (approximately 30ul) 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

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 Responsibility

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 applicable 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 applicable 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, Laboratory coat Micropipette

6.6.8 Storage And Stability

- i. If a delay is encountered in sample processing, store at 2-8°C for up to 72 hours is permissible.
- ii. CSF, plasma, serum may be stored for longer period at -20°C provided they are not repeatedly thawed and refrozen
- iii. Whole blood in transit should be maintained at 2-8°C not -20°C.

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 infectious.

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-mercaptoethanol 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 Responsibility

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) Assay diluent	Disposable gloves, Laboratory coat Micropipette

6.7.8 Storage And Stability

- a. The test device in the sealed pouch can be stored at 2-40⁰C 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 2-8⁰c for up 3 days
- c. Serum and plasma maybe stored at 2-8⁰c for up 7 days, for long term storage, serum and plasma specimens should be kept at -20⁰c 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

14.1 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 Responsibility

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 Laboratory coat 70% alcohol Mask

6.8.8 Storage And Stability

Store Covid 19 rapid kit device 2-30°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 infectious.
- 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 Documents

Sample collection manual

6.8.18 References

Manufacture package insert kit for SARS COV-2

ASOT Test Kit insert:

Atlas Medical,

CHAPTER SEVEN: BACTERIOLOGY

7.1 POTASSIUM HYDROXIDE (KOH) 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 Responsibility

Competent Health Laboratory Practitioners are responsible for implementing this test procedure.

The Head of Microbiology/who assigned 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 applicable

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 (ZN) 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 Responsibility

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 carbolfuscin, 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 processed within 3 days 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 infectious.

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). Manual 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 Responsibility

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 acid-alcohol. 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 processed 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 bacilli 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 (200magnification, one length 20x=field 200=HPF	Fluorescence (400magnification, 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 Responsibility

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
<ul style="list-style-type: none">• Crystal violet as primary stain• Lugol's iodine as mordant• 10% Acid/Acetone as decolourizer• Neutral red/safranin as counter stain	<ul style="list-style-type: none">• Gloves• Gauze• Waste bins• Grass slides• Applicator stick

7.4.8 Storage and Stability

- i. Samples are stable at 2-8°C for 7 days
- ii. store reagents as 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 infectious.

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 decolorisation 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: Author Monica Cherbourg 6th edition

7.5 DIAGNOSIS OF MTB/RIF TESTING USING A TRUENAT MACHINE

7.5.1 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 real-time 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.

7.5.2 Scope

This SOP describes the use of the Truenat™ MTB Plus assay, a chip-based Real-Time Polymerase Chain Reaction (PCR) test, for the semi-quantitative, detection and diagnosis of Mycobacterium tuberculosis complex bacteria (MTBC) in human sputum samples

7.5.3 Responsibility

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.

7.5.4 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 micro-PCR 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

7.5.5 Sample Requirements

Sputum sample type, collected as either Spot or morning sputum sample

7.5.6 Equipment

True prep AUTO Sample Prep device, Truelab Real Time micro PCR Analyser and Refrigerator

7.5.7 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	Truelab Duo Real Time Quantitative micro PCR Analyser	Truenat™ Positive Control Kit - Panel I Powder free disposable gloves	Liquefaction buffer Lysis buffer

Trueprep AUTO MTB Sample Pre-treatment Pack Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit	Truenat™ MTB Plus micro PCR chip Truelab™ micro PCR printer Truepet SPA fixed volume (6 µl) Precision micropipette • DNase and RNase-free pipette tips with filter barrier	Two waste disposal containers, with lids, containing bleach solutions Timer Two waste bags Micro tube Stand . Cartridge Holder Two cryovials racks	Conc. bleach and 70% alcohol
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Note: Connect a new reagent pack to the Trueprep Auto v2 device by inserting the Plug-in Connector into the slot provided.

7.5.8 Storage and Stability

store at room temperature for 3 days

Store at 2°C -8°C

7.5.9 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.

7.5.10 Calibration

Calibration is performed as per schedule

7.5.11 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.

7.5.12 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.”
- xii. 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.
- ii. Use a portion of the same DNA eluate to test for RIF resistance using a Truenat MTB-RIF Dx chip.
- iii. Start by returning to Step 3 in the PCR TB test process and repeat for RIF-resistance by Selecting “MTB RIF” as the test type in the Truelab micro-PCR Analyser.
- iv. RIF-resistance testing takes an additional 60 minute.

7.5.13 Biological Reference Interval

Not applicable

7.5.14 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

7.5.15 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.

7.5.16 Performance Characteristics

Refer to the method verification report of this procedure.

7.5.17 Supporting Document

Sample collection manual

7.5.18 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>

ANNEX 1: LIST OF PARTICIPANTS WHO DEVELOPED THE SOPs

SN	NAME	TITILE	FACILITY
1.	Adam Mwakyoma	Laboratory Scientist	Kilimanjaro Christian Medical Centre (KCMC), Moshi
2.	Amedeus Mushi	Laboratory Scientist & Epidemiologist	HLPC, MoH, Dodoma
3.	Anjela Maufi	Laboratory Scientist	Kitete RRH, Tabora
4.	Antusa Massawe	Laboratory Scientist	Dunga H/C, Tanga
5.	Baraka Mathias	Laboratory Scientist	Maranatha Hospital, Mbeya
6.	Betrand Msemwa	Microbiology & Immunologist	Catholic University of Health and Alliened Sciences (CUHAS), Mwanza
7.	Bezard Ngumbuchi	Quality Officer	PHLB, Dodoma
8.	Ceif Abdul	Quality Officer	NPHL, Dar es Salaam
9.	David Ocheng	Laboratory Expert/Consultant	Ilala, Dar es Salaam
10.	Desmond Kileo	ICTO	DHCTSU, MoH, Dodoma
11.	Dickson Charles Kakuntebe	Laboratory Scientist	Cardinal Rugambwa Hospital, Dar es Salaam
12.	Dominic Fwiling'afu	Registrar	PHLB, MoH, Dodoma
13.	Dr. Alex Magesa	DDS	DHCTSU, MoH, Dodoma
14.	Dr. Goodluck Tesha	Epidemiologist	Jhpiego, Dar es Salaam
15.	Dunstan Haule	Laboratory Quality Officer	NBTS-HQ, Dar es Salaam
16.	Edward Lushishi	Laboratory Technologist	Singida RRH, Singida
17.	Emmanuel Ndaki	Laboratory Scientist	NBTS Lake zone, Mwanza
18.	Eng. Gervas H. Swai	Biomedical Engineer	DHCTSU, MoH, Dodoma
19.	Eng. Suniva S. C. Haule	Head Health Care and Technical Services	DHCTSU, MoH, Dodoma
20.	Ezekiel Mng'ong'o	Laboratory Scientist	Maranatha Hospital, Mbeya
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SN	NAME	TITLE	FACILITY
22.	Ferdinand Matata	Head of Diagnostic Services	PORALG, Dodoma
23.	Frank Changala	Laboratory Scientist	NPHL, Dar es Salaam
24.	Dr Haji Hussein	Technical officer	ICAP, Dar es Salaam
25.	Frank Mushi	Laboratory Scientist	Manyara RRH, Manyara
26.	Frank Shemhande	Laboratory Quality Officer	SBNRH-Ndanda, Mtwara
27.	Godfrey Mahundi	Laboratory Manager	Dodoma MC Hospital
28.	Helman Mhangala	Deputy Laboratory Manager	NBTS Central Zone, Dodoma
29.	Ibrahim Mauki	Laboratory Scientist	NPHL, Dar es Salaam
30.	Idrissa Hassan	Laboratory Service Coordinator	PORALG, Dodoma
31.	Innocent A. Chinguile	Laboratory Scientist & Epidemiologist	Training, MoH, Dodoma
32.	Jackson Luvamunda	Quality Officer	Sinza HC, Dar es Salaam
33.	Jackson Zengo	Deputy National Laboratory Quality Officer	DHCTSU, MoH, Dodoma
34.	Jacob Lusekelo	National Laboratory Quality Officer	DHCTSU, MoH, Dodoma
35.	James Mziray	Laboratory Service Coordinator	PO-RALG, Dodoma
36.	Joakim Chacha	LS-HSEEP	DHCTSU, MoH, Dodoma
37.	Joyce Samson	Laboratory Quality Officer	Sekou-Toure RRH, Mwanza
38.	Kadogosa Saimon	Laboratory Technologist	Singida RRH, Singida
39.	Lanja Kahemela	Laboratory Quality Officer	MNH, Mloganzila, Dar es Salaam
40.	Maige Ngeleja	DSCMC	MoH, Dodoma
41.	Mary F. Mtui	Registrar	HLPC, MoH, Dodoma
42.	Mayala Lushina	Laboratory Quality Officer	MNH, Upanga, Dar es Salaam
43.	Michael Mazoya	Laboratory Scientist	NBTS, Dar es Salaam
44.	Mzelifa Daudi	Microbiologist	University of Dodoma (UDOM), Dodoma
45.	Pius R. Tarimo	Laboratory Scientist & Epidemiologist	Kilimanjaro Christian Medical University College (KCMUCo), Moshi
46.	Rajabu Muninge	Laboratory Scientist	Maranatha Hospital, Mbeya

SN	NAME	TITILE	FACILITY
47.	Rashid Nassoro	Laboratory Quality Officer	Morogoro RRH, Morogoro
48.	Reuben Abednego	Laboratory Scientist	NPHL, Dar es Salaam
49.	Reuben Lema	Deputy Laboratory Manager	Morogoro RRH, Morogoro
50.	Reuben S. Mkala	Ag. Head of Laboratory Service	DHCTSU, MoH, Dodoma
51.	Richard Kinyaha	Laboratory Scientist	Kibong'oto IDH, Kilimanjaro
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ANNEX 2: BIOLOGICAL REFERENCE INTERVALS FOR FULL BLOOD COUNT

Parameter	Reference Range					
	Adult		Infants		Children	SI Unit
	Males	Females	Birth	1 month	1 year	
WBC	4.00 - 10.0	4.0 - 10	10 - 26	5.0 - 9.0	6.0 - 16.0	$\times 10^3 / \mu\text{L}$
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	$\times 10^6 / \mu\text{L}$
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	$\times 10^3 / \mu\text{L}$
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	
Aspartate 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

Test Name	Normal range	SI Unit
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	mmol/L
Triglycerides	0 - 1.69	mmol/L
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

Test Name	Normal range	SI Unit
	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
T₃	1.45 - 3.48	pg/ml
CK-MB	0.0 - 6	%
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	mlu/mL
	25 for early pregnancy	mlu/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 10-28	pg/ML
	Male 11-44	pg/ML
Prolactin	Male	ng/ml

Test Name	Normal range	SI Unit
	3.46-19.40	ng/ml
	Female 5.18-26.53	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
	Luteal phase 0.90-9.33	ng/ml
	Postmenopausal	ng/ml
	10.39-64.57	ng/ml
FSH	Male 0.95-11.95	mlu/mL
	Follicular phase 3.03-8.08	mlu/mL
	Midcycle peak 2.55-16.69	mlu/mL
	Luteal phase 1.38-5.47	mlu/mL
	Postmenopausal	mlu/mL
	26.72-133.41	mlu/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/PANIC VALUES THAT CALLS 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
Bilirubin Total for new Born	Newborn 24hours ≥ 1374 umol/L 48hours ≥ 2224 umol/L 84hours ≥2904 umol/L 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/μl	



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