Adama Public Health Research and Referral Laboratory Center

LABORATORY HAND BOOK

	Name	Designation	Signature	Date
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REVISION AND AMENDMENT

A. Periodic Review of Document

Revision No	Review Date	Reviewed by:		Approved by:	
		Name	Signature	Name	Signature
5	July 16, 2021	Haile Benti		Daba Mulleta	

B. Version Change History/Description

Version No	Effective Date	Description of Version Change	Name & Signature of Reviewer	Name & Signature of Approval
4			Haile Benti	Daba Mulleta

C. Amendment

Proposed by	Section	Summary of Changes	Date of amendments

Quality manager	Quality manager office	1. Changed title from Clinician Hand Book to Laboratory Hand Book 2. Added information on sample requirements for liquid culture, LPA for TB section. 3. Added information on sample requirements for Uric acid and Total protein 4. Corrected reference ranges for Chemistry tests 5. Changed TAT for HIV DNA PCR, Viral Load and for Gene Expert tests	01/04/2008 E.C
Quality manager	Quality manager office		1/06/2009E.C
Quality Manager	Quality manager office	1. Name and logo of the organization changed. 2. The position 'Laboratory director' named as Chief Executive Officer (CEO) 3. Contact persons on page '***8 of 45***' has updated 4. Request forms are updated in the ***annex*** part	03/08/2011 EC

I. Acknowledgement

Adama Public Health Research and Referral Laboratory Center (APHRRLC) management would like express sincere gratitude to Oromia regional health bureau for its administrative and technical support, Ethiopian public health institute(EPHI), Center for Disease Control- Ethiopia (CDC-E) and partners for their technical and logistic support to ensure smooth flow of quality management system. The management would also like to acknowledge and recognize its entire staff for unreserved contribution and commitment to realize the accomplishment of this Laboratory Hand Book.

II. Table of contents

III. Foreword

This laboratory hand book has been prepared by Adama Public Health Research and Referral Laboratory Center (APHRRLC) for clinicians to use as a reference guide when seeking service and sending samples for analysis. The quality of patient test results provided by APHRRLC is largely dependent on quality of samples submitted. Therefore, clinicians and all specimen collection sites have a crucial role to play in ensuring quality service delivery hence quality and timely management of patients. This Laboratory hand book provides information on the scope of services offered by APHRRLC and guidelines on specimen requirements as well as transportation of samples.

APHRRLC welcomes any comments, suggestions and complaints from all who use our services. This will enhance our continuous improvement programmes which are aimed at providing services that meet international standards in APHRRLC diagnosis.

I hope you will find this information of value and trust that it will enable you to optimize your use of the services avail in APHRRLC.

Daba Mulleta

APHRRLC CEO

IV. Abbreviations

BSL	Bio safety Level
BRC	Bio Resource Center
CDC	Centers for Disease Control and Prevention
CBC	Complete Blood Count
CD4	Cluster of differentiation 4
DNA	Deoxyribonucleic Acid
DST	Drug susebtalblity test
EQA	External Quality Assessment
EPHI	Ethiopian Public Health Institute
ISO	International Organization for Standardization
IQC	Internal Quality Control
IT	Information Technology
LIS	Laboratory Information System
QC	Quality Control
QM	Quality Manual
QMS	Quality Management System
QSE	Quality System Essential
APHRRLC	Adama Public Health Research and Referral Laboratory Center
PCR	Polymerase Chain Reaction
RIF	Rifampicine
TAT	Turn- around Time
TI	Tran isolates media
KOH	Potassium Hydroxide
REQA	Regional External Quality Assessment
SOP	Standard operating procedure

V. Terms and definitions

Test: The intended test type required to be analyzed

Patient preparation: The requirements that the patient has to do before specimen collection.

Specimen type: The type of specimen to be collected. It should be the scientifically recommended or acceptable specimen for the analysis of the intended test.

Container: The appropriate means of holding the specimen for storage and transportation.

Specimen volume: The minimum amount of specimen required to successfully complete the test.

Transport conditions: These are the required conditions for transporting the sample. Example, at room temperature, refrigerated, screw caped, triple packed.

Storage condition and stability: when the test analysis is delayed, the specimen should be stored at the right or acceptable conditions. So the acceptable storage condition and the time how long can it stay stable (without specimen disintegration) at that condition should be indicated.

Turn Around Time (TAT): The duration from the time of receipt of the specimen at the reception to the time of report delivery to the patient or referring health facilities. Except on some extraordinary occasions, the result will be delivered as per the specified TAT.

Reference Range: These are the normal ranges of the target population. It is important to keep in mind that the reference range of a specific test may vary with test method i.e. we will communicate in case we change the test methods. Additionally, reference ranges may be specific for specific age and sex groups and type of specimen collection. For further information or references, you may contact the testing personnel.

Method: The analytical procedure that is used to conduct the test and detect the analyte.

Critical values: As these values are indicators for the critical status of the patient, results are communicated to the patient and/or ordering clinicians, immediately after the test are completed. The communication will be performed through phone-call or fax.

Cost: The amount a customer should expect to pay to APHRRLC for routine testing services. Costs can change based on specific requirements. Other APHRRLC service like ART monitoring, and TB culture service are free of charge.

1. Introduction

Adama Public Health Research and Referral Laboratory Center (APHRRLC) provides a high quality, cost-effective service to the public and private health facilities mainly within Oromia Regional State and is also serve as a referral center for other regional states. It is continually upgrading the test list offered to reflect medical development.

APHRRLC has implemented a quality management system in accordance with ISO 15189:2012 standard quality and competence of medical laboratories. This Laboratory hand book developed as partial fulfillment of the requirements of this standard.

All laboratory activities are performed with due care for the health and safety of staff and patients as well as proper care for the environment.

1.1. Permanent location

Adama Public Health Research and Referral Laboratory Center (APHRRLC) is located at:

Kebele 12

Adama Town

Oromia Region

Ethiopia

The postal address is P. O. Box: 688

Contact phone number +251-221-12-7962, +251- 221-11-0715 Fax +251-221-11-35-22

1.2. Contact information

Services /section	Contact person	Contact number
Laboratory CEO	Mr. Daba Mulleta	09-11-38-88-20
Quality Manager	Mr. Haile Benti	09-27-18-33-68
Referral Process Owner	Mr. Wake Abebe	09-11-31-39-25
Safety Officer	Mr. Abubeker Nura	09-11-70-72-90
Reception Focal Person	Mr.Issa Rabo	09-13-77-16-03
Clinical Bacteriology Focal Person	Mr. Ebissa Firdissa	09-11-75-22-11
Immuno Hematology Focal Person	Mr. Kiflu Itefa	09-13-04-75-78
TB Laboratory Focal Person	Mr. Ashenafi Eresso	09-20-96-77-88
Molecular Laboratory Focal Person	Mr.Olika Fekadu	09-13-35-65-38

1.3. Scope of services

Adama Public Health Research and Referral Laboratory Center quality management system covers all the activities performed in the laboratory which are:

2. Reception: Specimen collection, receiving, accessioning, storing, enter of test information using laboratory information system (LIS) and result collection and communication, monitoring of turn-around time (TAT).
3. Tuberculosis Laboratory: Smear microscopy(ZN/FM), GeneXpert, TB culture(solid and liquid) and DST (Line Probe Assay(LPA), phenotypic DST)
4. Clinical Chemistry Laboratory: GPT, GOT, Urea, Creatinine, Triglyceride, ALP, Cholesterol, Glucose. Bilirubin (Total & direct), Hormonal Assay and Immuno Assays
5. Immuno Hematology Laboratory: - CD4 count and complete blood cell count (CBC).
6. Clinical Bacteriology Laboratory: - Gram stain, culture and drug susceptibility test (DST), fungal wet mount, and KOH, urine microscopy.
7. Molecular Laboratory: HIV Viral Load, HIV DNA PCR, Human Papiloma Virus, SARS-Cov2 and Hepatitis B Virus Viral Load
8. Operational Research: Conduct and participate on operational research.
9. Outbreak investigation :- Participate on etiological agent identification and epidemiological outbreak investigation in collaboration with other stakeholders
10. **Surveillance**: Participate on the national HIV, meningitides sentinel surveillance, SARS cov2, Tuberculosis, Influenza, Antimicrobial Resistance (AMR), etc…

10.1. Opening hours

Working days

A full APHRRLC service is available from each department between 8:00 AM and 5:00 PM during weekdays but each section may have extended service times; please consult each section focal person for details.

Weekends and public holidays

Routine specimens for analysis are not acceptable during weekend. However, some special prior arrangements can be made for bacteriological investigation during outbreaks. Test requiring urgent analysis should be brought to the attention of the APHRRLC staff through telephone before submitting the samples.

Service during outbreak and surveillance

During any outbreak and surveillance, arrangements for specimen delivery must be made by public health officer or zonal/ward health office to APHRRLC CEO.

11. Specimen requirements, transportation and turn-around times

11.1. Clinical Chemistry (COBAS-6000)

Note: For all chemistry tests if the supplies are bought it costs and if supplied through program it is for free.

Alkaline Phosphates, ALP

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or serum-separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Special considerations / instructions	It may cause unreliable results to the patient with Waldenstrom's macroglobulinemia
Method	Kinetic
Reference range	64-306IU/L
Critical value	NA
Turnaround time	1 day
Cost	-------- ETB

Alanine Aminotransferase (ALT/SGPT)

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic
Reference range	0-41IU/L
Critical value	NA
Turnaround time	1 day
Cost	------- ETB

Asparate Aminotransferase (AST/SGOT)

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	UV Kinetic
Reference range	0-50 IU/L
Critical value	NA
Turnaround time	1day
Cost	------- ETB

Creatinine

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube

Volume	2 ml of serum or 3-5 ml of whole blood	
Transport	Serum should be transported using triple packaging technique.	
Rejection criteria	Haemolzed , lipamic, less than <2ml	
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day.	
Method	End point. Fixed point	
Reference range	Male	Female
	0.6-1.3mg/dl	0.5-0.93mg/dl
Critical value	NA	
Turnaround time	1 day	
Cost	------ ETB	

Urea/BUN

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic. Fixed point
Reference range	15-39mg/dl
Critical value	NA
Turnaround time	1 day
Cost	------ETB

Triglyceride

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day

Method	Kinetic, Fixed point
Reference range	0-199mg/dl
Critical value	NA
Turnaround time	1 day
Cost	-------- ETB

Glucose

Patient preparation	Fasting specimen recommended
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	If serum not separated within 30 minutes, than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic, Fixed point
Reference range	70-105mg/dl
Critical value	NA
Turnaround time	1 day
Cost	------ ETB

Cholesterol

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic/ Fixed point
Reference range	0-240mg/dl
Critical value	NA
Turnaround time	1 day
Cost	------- ETB

Bilirubin Total

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic/ Fixed point
Reference range	0.1-1 mg/dl
Critical value	NA
Turnaround time	1 day
Cost	--------ETB

Bilirubin Direct

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	Kinetic/ Fixed point
Reference range	0-0.2mg/dl
Critical value	NA
Turnaround time	1 day
Cost	--------ETB

Total protein

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube

Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	End point
Reference range	60-83g/dl
Critical value	NA
Turnaround time	1 day
Cost	---------ETB

Uric acid

Patient preparation	Not necessary
Specimen type	Serum
Container	Red-Stopper tube or Serum-Separator tube
Volume	2 ml of serum or 3-5 ml of whole blood
Transport	Serum should be transported using triple packaging technique.
Rejection criteria	Haemolysis, lipamic, less than <2ml
Storage and stability	Transport the sample immediately if not possible Keep serum in refrigerator 2-8°c for 1day
Method	End point
Reference range	2.6-7.2mg/dl
Critical value	NA
Turnaround time	1 day
Cost	--------ETB

11.2. Haematology

Complete Blood Count (CBC)

Patient preparation	Not necessary
Specimen type	Whole Blood
Container	Lavender-stopper (EDTA whole blood) tube
Volume	3-5 ml of whole blood
Transport	At room temperature using triple packaging technique.
Storage and stability	Transport the sample immediately if not possible Keep whole blood at room temperature for 8 hr.
Method	~~Automatic Sysmex KX-21N~~

Reference range	

Parameters	units	Ranges
WBC	µl	4.8-10.8 x10^3
RBC	µl	4.2- 6.1 x 10^6
HGB	g/dl	12.0 -18.0
HCT	%	37.0 - 52.0
MCV	fl	81.0- 99.0
MCH	pg	30.0-34.0
MCHC	g/dl	30.0-36.0
PLT	µl	140 - 440 x10^3
LYM	%	14.1-52.8
MXD	%	1.06 -5.9
NEUT	%	39.6 -78.4
LYM	µl	1.1 – 3.6 x10^3
MXD	µl	0.4-1.3 x10^3
NEUT	µl	1.9 -7.9 x10^3
RDW-SDRL	%	37.0-47.0
RDW-CVRL	fl	12.0-14.0
PDW	fl	9.4-18.1
MPV	fl	7.4-10.4
P-LCR	%	10.7-45.0

Critical value	HGB<7g/dl, WBC >25.0X10^3/µl , Platelet <20x10^3/µl or >1000x10^3/µl
Turnaround time	Four hrs
Cost	Free

11.3. CD4 TEST

Patient preparation	Not necessary
Specimen type	Whole Blood
Container	Lavender-stopper (EDTA whole blood) tube
Volume	3-5 ml of whole blood
Transport	At room temperature using triple packaging technique.
Rejection criteria	Clotted ,delayed mare than 72hrs, inappropriate anticoagulant
Storage and stability	Transport the sample immediately if not possible Keep whole blood at room temperature for 8 hr.
Method	FACS-Presto

Reference range	<table><tr><td>Analyte</td><td>Units</td><td>Range</td></tr><tr><td>T. lym% (CD3+/CD45+)</td><td>%</td><td>55-84</td></tr><tr><td>T. helper %(CD3+CD4+/CD45+)</td><td>%</td><td>32-68</td></tr><tr><td>CD3 Abs. count</td><td>Cells/ µl</td><td>1116 -1963</td></tr><tr><td>CD4 Abs. count</td><td>Cells/ µl</td><td>500-1300</td></tr><tr><td>CD4 45 Abs. count</td><td>Cells/ µl</td><td>NA</td></tr></table>
Critical value	NA
Turnaround time	Five hrs
Cost	Free

11.4. Molecular Laboratory

11.4.1. DNA-PCR for infant HIV Diagnoses

Patient preparation	Not necessary
Specimen type	Dried blood spot(DBS)
Container	Use What man 903 Dried Blood Spots (DBS) card with envelope
Volume	50-80 µl of whole blood or 3-5 circles of on a What man 903 DBS.
Transport	DBS specimen should be transported using the appropriate packaging technique by using sealable bag, humidity indictor and desicant to prevent moisture.
Rejection criteria	Clotted, < 3Spot, small size
Storage and stability	Transport the sample as soon as possible if not Keep sample at room temperature for 5 days and sample is stable for up to 90 days.
Method	Polymerase chain reaction (PCR) using Roche reagent
Reference range	NA
Critical volume	NA
Turnaround time	10 days
Cost	Free

11.4.2. Viral Load

Patient preparation	Not necessary
Specimen type	Plasma
Container	Lavender-stopper (EDTA whole blood) tube
Volume	3-5 ml of whole blood
Transport	Transport separated plasma at 2-8^{0}c
Rejection criteria	Unlabeled, without request paper , <1ml , Contaminated(turbid)
Storage and	Keep whole blood at room temperature for only 4 hours and separate plasma

stability	as soon as possible and store until analysis at -20°c for longer period of time.
Stability	Plasma is stable for 7 days at 2-8^{0}c or 1 year at -20^{0}c.
Method	Polymers Chan reaction (PCR) using Abbott m2000rt (RT-PCR)
Reference range	N/A
Critical value	NA
Turnaround time	14 days
Cost	Free

11.5. Tuberculosis

11.5.1. ZN/FM

11.5.1.1. Sediment sputum (from direct processed sample)

Patient preparation	N/A
Specimen	Sputum
Container	Sterile, wide mouth, unbreakable, leak proofed container (50ml falcon tube)
Volume	1-5ml
Transport	N/A
Storage and stability	It should be kept refrigerated (-20°c) and kept until result of culture dispatch.
Rejection criteria	N/A
Reference interval	Not applicable
Critical value	AFB positive
Method	Light Microscopy(ZN) and Florescence Microscopy
Turnaround time	Two day
Cost	Free

11.5.1.2. Gene Xpert

Patient preparation	Morning sputum is preferable and adequate orientation should give for the patient to collect adequate and good quality of sputum sample.
Specimen	Sputum
Container	Sterile, wide mouth, unbreakable, leak proofed container (50ml falcon tube)
Volume	3-5mL
Transport	As soon as possible with triple packaging system, If not kept refrigerated
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated (2-8°c) and could be sent within 3 days.
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled. Specimen contains food

	particle and blood. Inadequate specimen quantity Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	RIF resistance
Method	GeneXpert (Real-time PCR)
Turnaround time	8hrs
Cost	Free

11.5.1.3. CSF

Patient preparation	N/A
Specimen	CSF
Container	Sterile, wide mouth, unbreakable, leak proofed container(15ml falcon tube)
Volume	1-5mL
Transport	As soon as possible with triple packaging system
Storage and stability	Stable for 2 hr at room temperature. Do not refrigerate
Rejection criteria	Specimen delivered to the laboratory more than 2 hr after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity(less than 1 ml) Blood stained CSF sample
Reference interval	Not applicable
Critical value	MTB detected, RIF resistance
Method	GeneXpert (Real-time PCR)
Turnaround time	8hrs
Cost	Free

11.5.1.4. Lymph node and other tissues

Patient preparation	N/A
Specimen	Lymph node and other tissues
Container	Sterile, wide mouth, unbreakable, leak proofed container(50ml falcon tube)
Volume	N/A
Transport	As soon as possible with triple packaging, If not kept refrigerated at 2-8^{O}C
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated and could be sent within 3 days.
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled
Reference interval	Not applicable
Critical value	MTB Detected, RIF resistance
Method	GeneXpert (Real-time PCR)
Turnaround time	8hrs
Cost	Free

11.5.1.5. Other body fluids

Patient preparation	N/A
Specimen	Body fluid(pleural, peritoneal, synovial fluids)
Container	Sterile, wide mouth, unbreakable, leak proofed container(50ml falcon tube)
Volume	3-5mL
Transport	As soon as possible with triple packaging, If not kept refrigerated
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated and could be sent within 3 days
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	MTB Detected, RIF resistance
Method	GeneXpert (Real-time PCR)
Turnaround time	8hrs
Cost	Free

11.5.1.6. Culture

11.5.1.6.1. Sputum

Patient preparation	Morning sputum is preferable and adequate orientation should give for the patient to collect adequate and good quality of sputum sample.
Specimen type	Sputum
Container	Sterile, wide mouth, unbreakable, leak proofed container(50ml falcon tube)
Volume	3-5mL
Transport	As soon as possible with triple packaging, If not kept refrigerated
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated and could be sent within 3-5 days for culture.
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity. Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	Culture positive
Method	LJ (Solid media) and BACTEC MGIT 320 (Liquid media)
Turnaround time	65 days for solid culture and 42 days for liquid culture
Cost	Free

11.5.1.6.2. Laryngeal swab

Patient preparation	Before starting antimicrobial therapy (if possible). If drugs are administered, duration of treatment should be given in the request form. Collect early morning before food and drinks are taken
Specimen	Laryngeal swab
Container	Sterile absorbent cotton swab for collection and put in sterile test tube that has little saline solution (0.9%)
Volume	Wetted cotton swabs
Transport	As soon as possible with triple packaging. Refrigerate until transported
Storage and stability	Keep the laryngeal swab at 2-8°C and processed within one day
Rejection criteria	Specimen delivered to the laboratory more than one week after collection

	Specimen not labelled or wrongly labelled. Dry swab without visible evidence of tissue present.
Reference interval	Not applicable
Critical value	If MDR TB isolated and RIF resistance
Method	LJ (Solid media) and BACTEC MGIT 320 (Liquid media)
Turnaround time	65 days for solid culture and 42 days for liquid culture
Cost	Free

11.5.1.6.3. Aseptically collected body fluid

Patient preparation	Before starting antimicrobial therapy (if possible). If drugs are administered, duration of treatment should be given in the request form.
Specimen	Aseptically collected body fluid
Container	Sterile container with tight screw-capped seal, transparent and wide mouth tube (50ml falcon tube)
Volume	5-10 mL for whole blood, at least 3 mL for CSF and 20-50 mL for other body fluid
Transport	As soon as possible with triple packaging. Refrigerate until transported
Storage and stability	For fluids that may clot, sterile potassium oxalate (0.01-0.02ml of 10% neutral oxalate per ml fluid) or heparin (0.2mg per ml) should be added. Keep the body fluid at 2-8°C until processed
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	If MDR TB isolated RIF resistance
Method	LJ (Solid media) and BACTEC MGIT 320 (Liquid media)
Turnaround time	65 days for solid culture and 42 days for liquid culture
Cost	Free

11.5.1.6.4. Gastric lavage

Patient preparation	Before starting antimicrobial therapy (if possible). If drugs are administered, duration of treatment should be given in the request form. The collection should be made early in the morning with an empty stomach
Specimen	Gastric lavage
Container	Sterile container with tight screw-capped seal
Volume	5 -10 ml
Transport	Must be transported immediately to the laboratory. Keep in refrigerator

	before transportation
Storage and stability	Keep the gastric lavage at 2-8°C and processed within one day. It could be neutralized by adding 1 to 2 ml of disodium-hydrogen-phosphate solution to the gastric aspirate depending on the amount
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	NA
Method	LJ (Solid media) and BACTEC MGIT 320 (Liquid media)
Turnaround time	65 days for solid culture and 42 days for liquid culture
Cost	Free

11.5.2. DST

11.5.3. LPA (molecular)

Patient preparation	Morning sputum is preferable and adequate orientation should give for the patient to collect adequate and good volume of sputum sample.
Specimen type	Smear positive sputum and TB culture positive colony
Container	Sterile, wide mouth, unbreakable, leak proofed container(50ml falcon tube)
Volume	3-5mL and 2-3 colony
Transport	As soon as possible with triple packaging, If not kept refrigerated 2-8°c
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated and could be sent within 3-5 days
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity. Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	MDR TB and RIF resistance
Method	Line probe Assay / Molecular
Turnaround time	7 days for smear positive and 40-50 days for culture positive
Cost	Free

11.5.4. Conventional phenotypic DST

Patient preparation	Morning sputum is preferable and adequate orientation should give for the patient to collect adequate and good volume of sputum sample.
Specimen type	Sputum
Container	Sterile, wide mouth, unbreakable, leak proofed container(50ml falcon tube)
Volume	3-5mL
Transport	As soon as possible with triple packaging, If not kept refrigerated
Storage and stability	If it is not possible to send immediately, it should be kept refrigerated and could be sent within 3-5 days
Rejection criteria	Specimen delivered to the laboratory more than one week after collection. Specimen that is not labelled or wrongly labelled Inadequate specimen quantity. Specimen filled up to the lid of container resulting in leakages
Reference interval	Not applicable
Critical value	MDR TB and RIF resistance
Method	BACTE MGIT 320
Turnaround time	20-30 days
Cost	Free

11.6. Bacteriology

11.6.1. Blood culture

Patient preparation	Preferably, before administration of antibiotics and during febrile stage**.**
Sample	Whole Blood.
Container	Blood culture broth medium (directly inoculate).
Volume	5 ml of blood for children and ≥10ml injected in to 50ml TSY broth in duplicate at each episode for adults but in single tube for pediatric patients
Transport	Transported at room temperature (RT) as soon as possible.
Storage and stability	RT< 2hours to recover any organism.
Rejection Criteria	Delayed and unlabeled sample.
Reference range	N/A
Critical value	N/A
Turnaround time	7 days
Cost	50 ETB

11.6.2. Body fluids culture

Patient preparation	Preferably, before Administration of antibiotics.
Sample	Any body fluid except CSF
Container	syringe or blood culture broth (directly inoculate)
Volume	>5ml
Transport	Transported at room temperature (RT) as soon as possible.
Storage and stability	RT< 2hours to recover any organism.
Rejection Criteria	Delayed and unlabeled sample
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.3. CSF culture

Patient preparation	Preferably, before administration of antibiotics.
Sample	CSF
Container	Screw-cap tubes or TI (directly inoculate)
Volume	>1ml but 0.5ml sufficient when TI bottle used
Transport	Transported at room temperature (RT) as soon as possible.
Storage and stability	Up to 24 hours, at ambient temperature.
Rejection Criteria	Delayed, Refrigerated and unlabeled sample
Reference range	N/A
Critical value	All positive results
Turnaround time	4 days
Cost	50 ETB

11.6.4. Ear swab culture

Patient preparation	Preferably, before Administration of antibiotics.
Sample	Ear swab (middle or internal part)
Container	Sterile container with lid containing sterile swab
Volume	Two well wetted cotton swabs rolled on the affected part.
Transport	At room temperature (RT) or ice box should be transported to the APHRRLC immediately as much as possible.
Storage and stability	RT< 2hours to recover any organism.

Rejection criteria	Improper or non-sterile container, Delayed, unlabeled and dry swabs samples
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.5. Eye swab culture

Patient preparation	Preferably, before administration of antibiotics.
Specimen type	Eye swab
Container	Sterile container with lid containing sterile swab
Volume	Two well wetted cotton swabs rolled on the affected part.
Transport	At room temperature (RT) or Ice box should be transported as soon as possible.
Storage and stability	RT< 2hours to recover any organism.
Rejection Criteria	Improper or non-sterile container, Delayed, unlabeled and dry swabs samples
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.6. Genital culture

Patient preparation	Preferably, before administration of antibiotics and female patients should not wash their genital area.
Specimen type	Genital swab
Container	Sterile container with lid containing sterile swab
Volume	Two well wetted cotton swabs rolled on the affected part of Genital
Transport	Amies or Stuart's with Charcoal (Ice Box) should be transported as soon as possible.
Storage and stability	Amies or Stuart's media with Charcoal if >2 hr transport
Rejection Criteria	Improper or non-sterile container, delayed, unlabeled and dry swabs samples
Reference range	N/A
Critical value	N/A
Turnaround time	4 day

Cost	50 ETB

11.6.7. Pus culture

Patient preparation	Preferably, before administration of antibiotics**.**
Sample	Pus
Container	Sterile container with lid
Volume	Two well wetted cotton swabs rolled on the internal part of the wound
Transport	If not culturing for anaerobes- aspirate or swab in transport Stuart's or Amies transported media
Storage and stability	If less than 8hr at room temperature(RT) up to 24hrs in refrigerators for Non fastidious pathogens
Rejection criteria	Delayed and unlabeled sample
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.8. Sputum culture

Patient preparation	Preferably, before administration of antibiotics and morning sputum.
Specimen	sputum
Container	Sterile container with lid containing sterile swab
Volume	3-5ml
Transport	Streptococcus pneumonia dies within 2 hr.
Storage and stability	Keep refrigerated but do not expect to recover S. pneumonia
Rejection criteria	Improper or non-sterile container, Delayed and unlabeled sample
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.9. Stool culture

Patient preparation	Preferably, before administration of antibiotics
Sample	Stool
Container	Clean, dry, leak proof stool cup (container)

Volume	2-5 ml liquid or 2- 5 g solid.
Transport	Cary-Blair Salmonella and Shigella Buffered glycerol saline is best for Vibrio cholerae
Storage and stability	Cary-Blair (for Salmonella and Shigella) if >2 hr transport time
Rejection criteria	Improper or non-sterile container, delayed and unlabeled samples.
Reference range	N/A
Critical value	Vibrio cholerae
Turnaround time	3days
Cost	50 ETB

11.6.10. Throat culture

Patient preparation	preferably, before any antibiotics administration
Specimen type	Throat swab
Container	sterile container with lid containing sterile swab
Volume	Two well wetted cotton swabs rolled on the affected part of tonsil
Transport	Room temperature (RT)
Storage and stability	Group A streptococci survives drying well or use Stuart's media
Rejection criteria	Improper or non-sterile container, delayed, unlabeled samples.
Reference range	N/A
Critical value	N/A
Turnaround time	4 days
Cost	50 ETB

11.6.11. Urine culture

Patient preparation	Before any anti biotic administration and clean-catch morning urine is preferable.
Specimen type	Urine
Container	sterile container with lid (urine cup)
Volume	10ml
Transport	Refrigerate if >2 hr transport, Special boric acid tubes can be used if avail at APHRRLC.
Storage and stability	Stability $\leq$ 2 hours at RT un preserved, if preserved and stored at 4-6 ^{o}c, stable for $\leq$24 hours
Rejection criteria	Foley catheter , from catheterized patient bag ,delayed and un labelled samples
Reference range	N/A

Critical value	N/A
Turnaround time	3 days
Cost	50 ETB

11.7. Instructions for completing the request form

APHRRLC has request forms for ART monitoring, TB and Clinical bacteriology and used request forms prepared by Federal Ministry of Health for HIV DNA PCR. Clinicians and health facilities requesting investigations are required to use only these forms and ensure that all spaces provided are filled in completely.

The HIV DNA PCR request form expected to be completed in triplicate. The original and second copy should be send to the APHRRLC with the specimen. The third copy should be retained in the health facility. After laboratory test, the original form with completed results will be returned to the facility ART clinic and the copy will be retained in the laboratory.

NB: Relevant clinical information appropriate to the test(s) requested must be supplied e.g. history of administration of drugs, antenatal history, blood transfusion history etc. This information helps laboratory experts when they verify results before release. The minimum clinical information supplied relevant to the patient must include gender and age for interpretative purposes. A clear indication as to whether the tests requested are urgent or routine.

11.8. Specimen reception and rejection criteria

The following criterion is used to reject samples:

a) Incomplete identification information on the request form
b) Incomplete or wrongly labeled specimens
c) No request/requisition form accompanying the sample
d) Insufficient quantity
e) Wrong tube/specimen type for the test requested
f) Specimen container leaking, damaged or broken
g) Specimen not transported properly e.g. on ice if appropriate
h) Specimen clotted

i) Specimen haemolyzed
j) Specimen contaminated

NB: APHRRLC will not reject irreplaceable or critical specimens like cerebro-spinal fluid and neonate blood specimens. Such specimens are processed and put a note on comment while the laboratory staff contacts the clinician collecting the primary specimen to resolve the problem.

11.9. Verbal Requests

APHRRLC accepts verbal requests additional for tests on specimens already submitted. When a verbal request is received, a laboratory expert first checks if the specimen is still available and suitable to perform the additional test/s. The clinician is then advice to make a formal request while the specimen is being processed. In the meantime the laboratory expert fill-in the verbal request forms and sends it to the respective section. The results are only released when the formal request has been received in the APHRRLC.

11.10. Handling urgent specimens

APHRRLC ensures that all specimens labeled as 'urgent' are given due attention and processed quickly. In order to facilitate this process, samples must clearly be marked as "urgent" and or health facilities must communicate with the laboratory before delivering specimens for necessary preparations to be done. Following processing of the urgent specimens, APHRRLC staff will call the requesting clinician/health facility with the results; therefore clinicians are encouraged to always provide their contact numbers.

11.11. Advisory service

The management of APHRRLC has authorized its technical staff to offer customers with advice regarding correct specimen collection, handling and transportation to ensure that results produced are reliable. Technical staff can also offer advice on proper interpretation of patient results. Clinicians and health facilities are advised to use the contact details in this handbook to ask for any information regarding the laboratory's service from the relevant staff.

11.12. APHRRLCs policy on protection of confidential information

All staff of APHRRLC has signed a confidentiality undertaking and is bound not to disclose any patient information to anyone other than the requesting clinician. Any violation of this undertaking will result in disciplinary action.

11.13. APHRRLC complaints procedure

Any users of APHRRLC services wishing to raise a complaint against APHRRLC are required to do so following this procedure:

a) APHRRLC users can send their complaints by means of telephone calls (numbers provided in this handbook), e-mail, and suggestion box (at the laboratory's reception) or customer satisfaction surveys (which is conducted bi-annually by the laboratory).
b) For issues requiring immediate attention, health facilities or clinicians can contact the laboratory CEO on +251-221-12-7962 or +251- 221-11-0715.
c) Users who wish to receive feedback on their complaints in person should put their contact details; otherwise they can choose to remain anonymous.
d) After receiving feedback, the quality manager records all complaints on the complaints form and attaches the original copy of complaints where applicable.
e) The quality manager takes the complaints to the laboratory's CEO within 48 working hours of receiving the complaint and together they initiate investigations.
f) If the complain is such that it affects or has already affected patient/s results, the laboratory CEO orders work to be stopped in the affected area and informs the quality manager to record the complain as a major non-conformity.
g) Corrective action which includes establishing root cause of the problem is conducted as per procedures.
h) Target date for completion of corrective action is agreed upon by the laboratory CEO, quality manager and any staff assigned to do investigations.
i) Whether corrective action has been completed or not, the quality manager shall communicate with the complainant within 3 working days, acknowledging receiving the

complain and informing on the status of corrective action. This communication will not be possible where contact details are not provided.

12. Reference

1. Adama Public Health Research and Referral Laboratory Center (APHRRLC) Quality Manual Version-3 Nov.2007 E.C
2. Adama Public Health Research and Referral Laboratory Center System SOP Nov.2007 E.C
3. Adama Public Health Research and Referral Laboratory Center Technical SOPs, Nov.2007 E.C
4. Adama Public Health Research and Referral Laboratory Center System formats Nov.2007 E.C
5. Adama Public Health Research and Referral Laboratory Center Safety manual Version-3 Dec.2007 E.C
6. Adama Public Health Research and Referral Laboratory Center Technical formats Nov.2007 E.C
7. Laboratory reference range Study ,BCH, Adult values,1996 HEIA 696
8. Human reagent Kit insert
9. Hematological practice 4th ,edition ,2005
10. http://www.pubinfo.vcu.edu/pathlabs/print_menu/appendix_hematology_reference_ranges.pd
11. Adult values established by reference range studies performed by VCU Medical Center Hematology Laboratory
12. F.A. Davis , Hematology in practice ,2007
13. Dr.Aster Tsegaye,* Dr.Tsehaynesh Messele, Tesfaye Tilahun, Ermias Hailu, Tefera Sahlu, Ronan Doorly, Arnaud L. Fontanet, and Tobias F. Rinke de Wit Immunohematological Reference Ranges for Adult Ethiopians

13. Appendix

13.1. A.R.T Laboratory test request form

ADAMA PUBLIC HEALTH RESEARCH & REFERRAL LABORATORY CENTER

ADAMA, ETHIOPIA

Address: P.O. Box 688 Adama Tele: +251-22-112-7962 Fax: +251-22-111-3522 oregionallab@gmail.com

A.R.T LABORATORY TEST REQUEST FORM

Referring health facility: .....
Patient card number: .....
Age: ..... Sex: .....Unique ART number: .....
Specimen ID.....
Patient address: .....Tel.....

Ordering clinician: .....
Phone: clinician..... HF .....
WHO staging and clinical relevant information: .....

Type of specimen: .....
Specimen collection date: ..... Time: .....
Collected by: .....
Date of specimen received .....Time.....

Test Requested: ☐ Routine ☐ Urgent
CD4 count ☐ CBC ☐ Chemistry ☐
Serology ☐ Viral load ☐

Cost: ☐ Free ☐ Paid .....Et.birr Receipt number .....
(For lab use only) **LABORATORY REPORT** Reception Specimen ID.....

CBC(COMPLETE BLOOD COUNT)		
CBC & Differential	Result	Ref. Range*
WBC	µl	4.8-10.8 x10^3
RBC	µl	4.2- 6.1 x 10^6
HGB	g/dl	12.0-18.0
HCT	%	37.0- 52.0
MCV	%	81- 99
MCH	pg	30-34
MCHC	g/dl	30 -36
PLT	µl	140 - 440 x10^3
LYM%	%	14.1-52.8
MXD%	%	1.06 -5.9
NEUT%	%	39.6-78.4
LYM#	µl	1.1-3.6 x10^3
MXD#	µl	0.4 -1.3 x10^3
NEUT#	µl	1.9-7.9 x10^3
RDW-SDRL	%	37- 47
RDW-CVRL	fl	12-14
PDW	fl	9.4 -18.1
MPV	fl	7.4 -10.4
P-LCR	%	10.7- 45.0
Tech	Date	

CD4 COUNT	Result	Ref. range*
T.lym%(CD3+/CD45+)	%	55-84
CD3 Abs count	Cells/µl	1116-1962
T.helper%(CD3$^+$CD4$^+$/CD45$^+$)	%	32-68
CD4 Abs count(CD3$^+$CD4$^+$)	Cells/µl	500-1300
Lym. (CD45+)Abs count	Cells/µl	NA
Tech	Date	

CHEMISTRY		
Test	Result	Ref. Range *
ALP	IU/L	40-150
ALT(SGPT)	IU/L	0-50
AST(SGOT)	IU/L	0-60
Creatinine	mg/dl	0.6-1.1
B.U.N/urea	mg/dl	5-18
Triglyceride	mg/dl	0-200
Glucose	mg/dl	70-105
Cholesterol	mg/dl	0-220
Bilirubin T	mg/dl	0.1-1.2
Bilirubin D	mg/dl	< 0.2
Tech	Date	

VIRAL LOAD (RNA QUANTITATIVE TEST)
.....RNA Copies/ml
.....Lower detection limit <40 copies /ml
☐ Undetectable ☐ Not done
Detection Method: Real Time PCR
Tech ..... Date .....

*Reference range are for adults only

Comment.....
.....
.....
.....
.....
.....
.....

Test result verified by : Signature Date ...../...../.....E.C

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13.2. TB Laboratory test request form

ADAMA PUBLIC HEALTH RESEARCH & REFERRAL LABORATORY CENTER

ADAMA, ETHIOPIA

Address:P.O. Box 688 Adama Tele: +251-22-112-7962 Fax: +251-22-111-3522 oregionallab@gmail.com

TB LABORATORY TEST REQUEST FORM

Referring health facility: .....
Patient full name: .....
Age: ..... Sex: ..... Card number: .....
Specimen ID.....
Address: Region .....Zone.....
Woreda..... kebele.....
House No.....Tel. pat .....

Ordering clinician: .....
Phone: clinician..... HF .....
Patient TB registration No.....
Co-infection ☐ No ☐ Yes
Clinical relevant information: .....
.....
.....

TB disease type and treatment history; Site ☐ Pulmonary ☐ Exra pulmonary (specify) .....
Registration group: First line ☐ New ☐ Relapse ☐ Lost to follow up ☐ After failure of 1st treatment
☐ After failure of re- treatment ☐ MDR TB contact ☐ Other(previously treated with unknown)
Privious TB drug use: ☐ First Line ☐ Second Line
Resean for request : ☐ Diagnosis ☐ Follow up at .....months **during** treatment
☐ Follow up at .....months **after** treatment

Type of specimen: .....
Specimen collection date: ..... Time: .....
Collected by: .....
Date of specimen received .....Time.....

Test Requested: ☐ Routine ☐ Urgent
Microscopy ☐ TB culture only
GeneXpert
TB culture and DST (LPA)

Cost: ☐ Free ☐ Paid: ..... Et.Birr. Receipt number .....
(For lab use only) **LABORATORY REPORT** Reception Specimen ID.....

Microscopy result								
Method	**Microscopy Result**					Smear type		Remark
	Negative	Actual no	1+	2+	3+	Direct	Concentrated	
☐ Ziehl-Neelsen								
☐ Fluorescence								

GeneXpert result							
☐ MTB not detected ☐ Invalid ☐ No result ☐ Error							
☐ MTB detected, Rifampicin: ☐ Sensitive ☐ Resistant ☐ Indeterminate							
TB culture and Drug susceptibility testing(DST)							
Culture result			Positive ,Mycobacterium tuberculosis complex(MTBC)				
Contaminated	Negative	Non-TB bacteria	<50colonies Actual count	50-100 colonies- 1+	100-200 colonies -2+	200-500 colonies -3+	>500 colonies- 4+
Drug susceptibility test result (LPA)			Rifampicin				
			Isoniazid				
Comment							

Test done by :	Signature	Date ...../...../.....E.C
Final report verified by :	Signature	Date ...../...../.....E.C

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13.3. Clinical bacteriology laboratory test request form

ADAMA PUBLIC HEALTH RESEARCH & REFERRAL LABORATORY CENTER

ADAMA, ETHIOPIA

Address: P.O. Box 688 Adama Tele: +251-22-112-7962 Fax: +251-22-111-3522 oregionallab@gmail.com

CLINICAL BACTERIOLOGY TEST REQUEST FORM

Referring Health facility: .....
Patient full name: .....
Age ..... Sex: .....Pt. Card number: .....
Specimen ID.....
Patient address: .....
Telephone: Patient .....

Ordering clinician: .....
Phone: clinician..... HF .....
Diagnosis: .....
Current Antibiotics: .....
Clinical History: .....
.....

Type of Specimen: .....
Specimen Collection Date: .....Time: .....
Collected by: .....
Date of specimen received: ..... Time: .....

Test Requested: ☐ Routine ☐ Urgent
☐ Gram Stain ☐ Routine Culture and DST
☐ Acid-fast Stain:
Others: .....

Cost: ☐ Free ☐ Paid.....Et.birr
(For lab use only)**LABORATORY REPORT**

Receipt number .....
Reception Specimen ID. .....

Gram stain:
Polymorphonuclear neutrophils (PMN): .....
Squamous epithelial cells: .....
Gram-positive cocci: .....
Gram-negative rods: ..... Yeast: .....
Others: .....
India ink: .....
Acid-fast stain: .....
Tech: ..... Date: .....

Culture (Preliminary report): ....
.....
.....
Tech: .....Date: .....

Culture (Final report): .....
.....
.....
Tech: .....Date: .....

Drug	Zone size (mm)	Interp. (S, I, R)	Drug	Zone size (mm)	Interp. (S, I, R)
Ampicillin			Ampicillin		
Cefazolin			Cefazolin		
Gentamicin			Gentamicin		
Amoxicillin/clavulanate			Amoxicillin/clavulanate		
Piperacillin-Tazobactam			Piperacillin-Tazobactam		
Cefuroxime			Cefuroxime		
Cefotaxime			Cefotaxime		
Ciprofloxacin			Ciprofloxacin		
Imipenem			Imipenem		
Trimethoprim/Sulfa			Trimethoprim/Sulfa		
Chloramphenicol			Chloramphenicol		
Nitrofurantoin			Nitrofurantoin		

Final report verified by: ..... Date: .....

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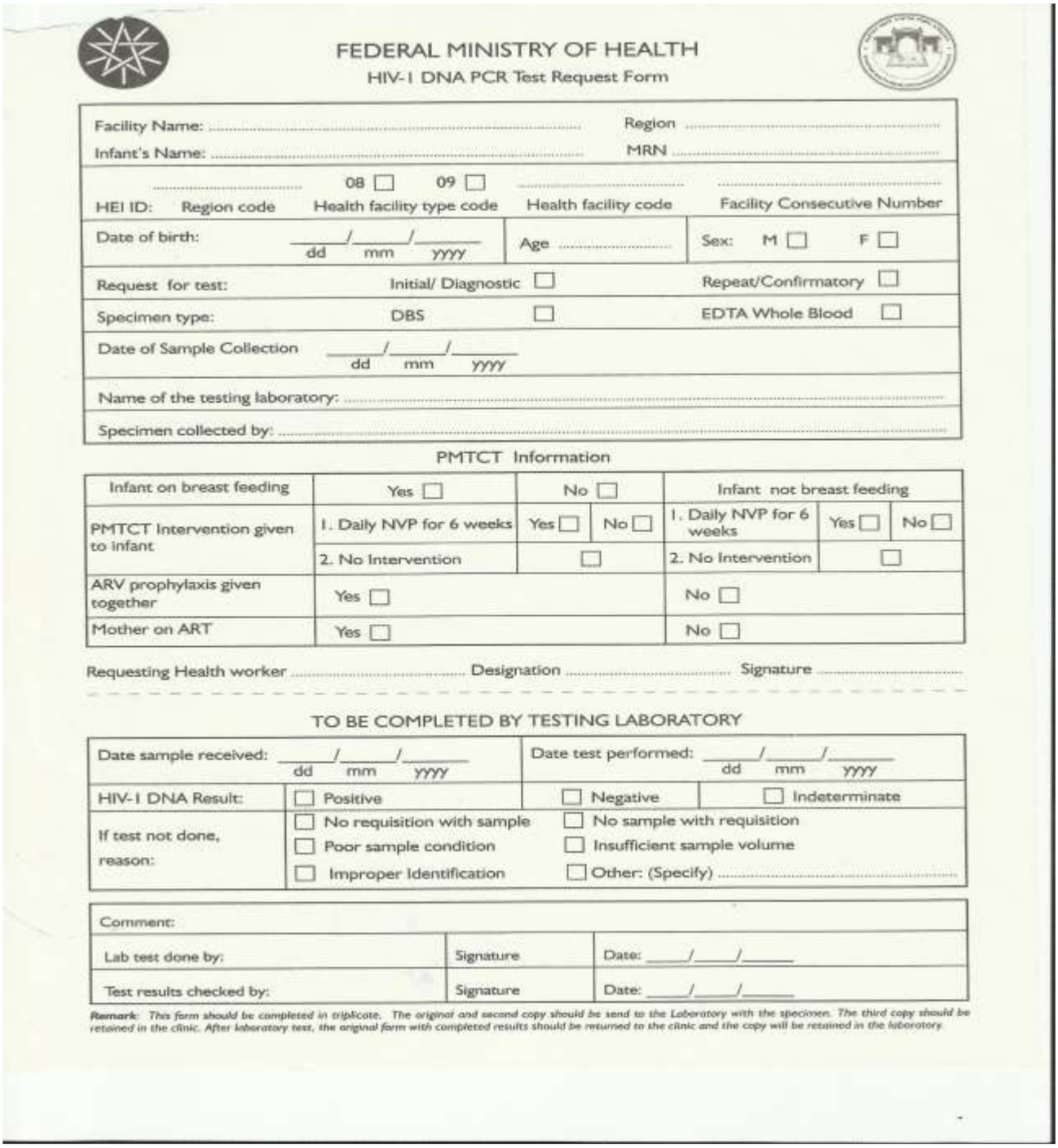
13.4. HIV-1 DNA PCR test request form



FEDERAL MINISTRY OF HEALTH

HIV-1 DNA PCR Test Request Form

Facility Name: ..... Region .....

Infant's Name: ..... MRN .....

HEI ID: ..... Region code: 08 ☐ 09 ☐ Health facility type code: ..... Health facility code: ..... Facility Consecutive Number: .....

Date of birth: ____/____/____ (dd/mm/yyyy) Age ..... Sex: M ☐ F ☐

Request for test: Initial/ Diagnostic ☐ Repeat/Confirmatory ☐

Specimen type: DBS ☐ EDTA Whole Blood ☐

Date of Sample Collection ____/____/____ (dd/mm/yyyy)

Name of the testing laboratory: .....

Specimen collected by: .....

PMTCT Information

Infant on breast feeding	Yes ☐	No ☐	Infant not breast feeding		
PMTCT Intervention given to infant	1. Daily NVP for 6 weeks	Yes ☐ No ☐	1. Daily NVP for 6 weeks	Yes ☐	No ☐
	2. No Intervention	☐	2. No Intervention	☐	
ARV prophylaxis given together	Yes ☐		No ☐		
Mother on ART	Yes ☐		No ☐		

Requesting Health worker ..... Designation ..... Signature .....

TO BE COMPLETED BY TESTING LABORATORY

Date sample received: ____/____/____ (dd/mm/yyyy)		Date test performed: ____/____/____ (dd/mm/yyyy)	
HIV-1 DNA Result:	☐ Positive	☐ Negative	☐ Indeterminate
If test not done, reason:	☐ No requisition with sample	☐ No sample with requisition	
	☐ Poor sample condition	☐ Insufficient sample volume	
	☐ Improper Identification	☐ Other: (Specify)	

Comment:		
Lab test done by:	Signature	Date: ____/____/____
Test results checked by:	Signature	Date: ____/____/____

Remark: This form should be completed in triplicate. The original and second copy should be send to the Laboratory with the specimen. The third copy should be retained in the clinic. After laboratory test, the original form with completed results should be returned to the clinic and the copy will be retained in the laboratory.

13.5. National viral load request form

Federal Ministry of Health, Ethiopia
Laboratory Requisition and Report form for HIV Viral Load Testing

1. Health Facility Information

Facility Name: _____ Facility Code: _____ Tel.No._____

Region: _____ District:_____

Requested by: Name _____Signature:_____ Date (ET)__/__/____ (dd/mm/yyyy)

2. Client Information

Unique ART ID: __/__/__/__/__/__/__/__/__/__/__ MRN __/__/___/___/__/__

Sex: ❑ M ❑ F

Age (years) _____ <1 year (in months) __/__/

3. Current ART regimen

❑Adult First Line Regimen:_____

Date (ET) Initiated __/__/____ (dd/mm/yyyy)

❑ *If Pediatric*, First Line Regimen: _____

Date (ET) Initiated __/__/____ (dd/mm/yyyy)

❑ Current Second Line Regimen:_____

Date (ET) Initiated __/__/____ (dd/mm/yyyy)

4. ART Adherence

❑ Good >95%

❑ Fair (85-94%)

❑ Poor <85%

5. Is the client pregnant ❑Yes or ❑No **Breastfeeding** ❑Yes or ❑No

6. CD4 count history (CD4% for <5 years)

Most recent result_____cells/ul Date (ET) __/__/____ (dd/mm/yyyy)

Baseline result (pre ART) _____ cells/ul Date (ET) __/__/____ (dd/mm/yyyy)

7. Current Clinical observations/symptoms:

WHO (Treatment) Staging : ❑ I ❑ II ❑ III ❑ IV

8. Reason for Test

Routine viral load: ❑ First viral load test (6 months or more post ART) ❑ Annual Viral Load (VL) Test

Suspected ART Failure: ❑ Initial Viral load >1000 copies/ml (repeat) ❑Immunological ❑Clinical

9. To be filled by referring laboratory

Date (ET) Specimen Collected: __/__/____ (dd/mm/yyyy) Time (ET):_____

Specimen type
❑ Whole Blood ❑ DBS ❑ Plasma
Date specimen sent to Reference laboratory __/__/____ (dd/mm/yyyy Time (ET):_____

10. For Testing Laboratory use only

LAB ID: [ ]
Date Received (ET) __/__/__ __(dd/mm/yyyy)
Specimen quality
❑ Acceptable ❑ Unacceptable
Reason_____

Test results:

Test Date (ET): __/__/____(dd/mm/yyyy)

Test result: _____copies/ml

Tested by _____Signature_____

Dispatch date (ET)____/___/_____ (dd/mm/yyyy)

Reviewed by_____Signature_____

13.6. Vacutainer colour system

Vacutainer® Tubes With		Additive	Mix Number of Times	Use
Hemogar™ Closure	Vacutainer ®Tubes with Conventional Stopper			
 Gold	 Red/Grey	Clot activator and gel for serum separation	5	For serum determinations in chemistry. May be used for routine blood donor screening and diagnostic testing of serum for infectious disease. Tube inversions ensure mixing of clot activator with blood. Blood clotting time: 30 minutes.
 Light Green	 Green/Grey	Lithium heparin and gel for plasma separation	8	For plasma determinations in chemistry. Tube inversions ensure mixing of anticoagulant (heparin) with blood to prevent clotting.
 Red	 Red	Silicone coated (glass) • Clot activator, Silicone coated (plastic	0-5	For serum determinations in chemistry. May be used for routine blood donor screening and diagnostic testing of serum for infectious disease. Tube inversions ensure mixing of clot activator with blood. Blood clotting time: 60 minutes
 Green	 Green	• Sodium heparin •Lithium heparin	8	For plasma determinations in chemistry. Tube inversions ensure mixing of anticoagulant (heparin) with blood to prevent clotting.

 Gray	 Gray	Potassium oxalate/ sodium fluoride • Sodium fluoride/Na2 EDTA • Sodium fluoride (serum tube	8	For glucose determinations. Oxalate and EDTA anticoagulants will give plasma samples. Sodium fluoride is the antiglycolytic agent. Tube inversions ensure proper mixing of additive with blood.
 Lavender	 Lavender	• Liquid K3EDTA (glass) • Spray-coated K2EDTA (plastic)	8	K2EDTA and K3EDTA for whole blood hematology determinations. K2EDTA may be used for routine immunohematology testing, and blood donor screening. Tube inversions ensure mixing of anticoagulant (EDTA) with blood to prevent clotting
 White		• K2EDTA and gel for plasma separation	8	For use in molecular diagnostic test methods (such as, but not limited to, polymerase chain reaction [PCR] and/or branched DNA [bDNA] Amplification techniques). Tube inversions ensure mixing of anticoagulant (EDTA) with Blood to prevent clotting.
 Light Blue	 Light Blue	• Buffered sodium citrate0.105 M (≈3.2%) glass 0.109 M (3.2%) plastic • Citrate, theophylline, adenosine, dipyridamole (CTAD)	8	For coagulation determinations. CTAD for selected platelet function assays and routine coagulation determination. Tube inversions ensure mixing of anticoagulant (citrate) to prevent clotting.

13.7. Procedure for specimen collection

A. **Practice universal precautions:**

- Wear gloves when handling blood/body fluids.
- Change gloves after each patient or when contaminated.
- Wash hands frequently.
- Dispose of items in appropriate containers.
- Dispose of needles immediately upon removal from the patient's.
- Clean up any blood spills with a freshly made 1:10 bleach disinfectant.

B. Equipment needed

- The following are needed for routine vein puncture

  Evacuated collection tubes – the tubes are designed to fill a predetermined volume of blood by vacuum. The rubber stoppers are colour coded according to the additive the tube contains. Blood should NEVER be poured from one tube to another since the tube can have different additives or coatings.

- Needles – The gauge number indicates the bore size: the larger the gauge number, the smaller the needle bore.
- Holder – use with the evacuated system.
- Tourniquet – wipe off with alcohol and replace frequently.
- Alcohol wipes –70% isopropyl alcohol.
- Adhesive bandages / tape – protects the vein puncture site after collection.
- Needle disposal unit – needles should NEVER be broken, bent, or recapped. Needles should be placed in disposal unit IMMEDIATELY after their use.
- Gloves – can be made of latex, rubber, or vinyl, and are worn to protect the patient and the phlebotomist.
- Syringes – may be used in place of the evacuated collection tube for special circumstances.

C. Procedure for vein selection:

- The median cubital and cephalic veins of the arm are used most frequently.

- Palpate and trace the path of veins with the index finger. Arteries pulsate, are most elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord like, and roll easily.
- If superficial veins are not readily apparent, you can force blood into the vein by massaging the arm from wrist to elbow, tap the site with the index and second finger, apply warm, damp washcloths to the site for 5 minutes, or lower the extremity to allow the veins to fill.

D. Drawing the blood

- Position the patient so he or she is comfortable and safe in case the patient becomes faint and falls.
- Recommended needle size: 20G, 21G or 22G.
- Closed vacutainer system is recommended.
- Select tube or tubes appropriate for type of samples desired.
- Select site for vein puncture.
- Put on gloves.
- Prepare vein puncture site with alcohol prep. Cleanse in a circular fashion, beginning at the center and working outward.
- Do not palpate vein puncture area after cleansing. Allow site to dry.
- Apply the tourniquet 3-4 inches above the selected puncture site. Do not place too tightly or leave on more than 2 minutes.
- Remove needle shield. Perform vein puncture with patient's arm in a downward position and tube stopper uppermost. this reduces the risk of backflow of any anticoagulant into the patient's circulation
- Push the tube onto the needle, puncturing the stopper.
- Remove tourniquet as soon as blood appears in tube, within 2 minutes of vein puncture. Do not allow contents of tube to contact the stopper during the procedure.
- When first tube has filled to its stated volume, remove it from the holder.
- Place succeeding tube in holder puncturing stopper to initiate flow.

- While each successive tube is filling invert previous tube GENTLY 5 times.
  DO NOT SHAKE. Vigorous mixing can cause haemolysis.
- When all tubes of blood have been collected, remove the last tube from the vacutainer holder, place a cotton ball or gauze over the site and withdraw the needle in a smooth and cautious manner so as not to bruise the vein.
- After withdrawing the needle fully, apply pressure to the cotton ball over the puncture site and hold pressure. If patient is able ask them to apply pressure for 3 to 5 minutes until the bleeding stops.
- Discard the needle of the vacutainer into the biohazard container without re-capping the needle.
- Immediately invert the last tube GENTLY 5 times.

E. **Special preparation of patients before sample collection**

I. **Urine for culture**

Please avoid touching the inside of the container and /or the lid in order to maintain sterility

Female: The patient should be seated on the toilet or bedpan with legs separated. Separate labia minora (inner folds), cleanse opening of urethra three times with cleansing pads. Allow the initial stream of urine to pass, and then collect urine into a sterile container.

Male: cleanse glans three times with cleaning pads. Allow the initial stream of urine to pass, and then collect urine into a sterile container.

Please label the specimen with patient's name, date and time of collection.

The specimen should be submitted in the laboratory as soon as possible. If there is a delay, the specimen should be refrigerated.

II. **Sputum for culture**

1. Patient should rinse mouth and gargle with water immediately prior to collection.
2. Collect specimen from deep cough into a sterile container.
3. Patient should avoid any contamination with saliva.

4. Return specimen as soon as possible. If there is a delay, specimen should be refrigerated.
5. Label the specimen container with patient's name, date and time.

I have read, understood and agree to follow the Laboratory Hand Book as documented:

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