

PRIMARY SAMPLE COLLECTION MANUAL



OF

DEPARTMENT OF MICROBIOLOGY

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**DEPARTMENT OF MICROBIOLOGY,
K.G. Medical University, Lucknow**



DOC. NO.	TITLE	ISSUE NO.
KG/Micro/03	Primary Sample Collection Manual	02
Effective Date: 26/02/2022		

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AMENDMENT SHEET

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GENERAL INFORMATION

LABORATORY WORKING HOURS

The working hours, for the various divisions and specimen acceptance timings are provided in the tables below.

S. No.	Working Days	Working Hours
01	Monday- Saturday	09:00 am- 04:00 pm
02	Sundays & Holidays	10:00am- 12:30 pm

1.2 SPECIMEN ACCEPTANCE TIMINGS

	Timing	
OPD patients	9.00 a.m. – 2.p.m.	OPD counter
Indoor patients	9.00 a.m. – 2. p.m.	IPD counter
	2.p.m to 9.00 a. m on working days Round the clock on holidays	Urgent specimen will be received in Emergency Laboratory

1.3 LIST OF AVAILABLE LABORATORY EXAMINATIONS

ANNEXURE I

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1.0 Purpose

This manual is designed to provide users of the clinical testing services of the Department of Microbiology, KGMU, with information on the proper collection and handling of primary samples destined for testing in the laboratory.

2.0 Scope: It is intended as a quick reference guide for users of the Department of Microbiology Services both from within the hospital, and those from the community.

3.0 Responsibility

The Laboratory Director (LD), Department of Microbiology decides on the scope of clinical services provided by the department. The final decisions on sample acceptance/rejection rest with the laboratory director/designee.

4.0 Abbreviations

LD: lab Director

KGMU: King George's Medical University

HIV: Human Immunodeficiency Virus

TB: Tuberculosis

IPD: In door patient

OPD: Out Door patient

CSF: Cerebral Spinal Fluid

OT: Operation Theater

5.0 Procedure

General instructions

1. Use universal precautions for collecting and handling all specimens. In case a needle stick injury is sustained while collecting samples, follow standard post exposure prophylaxis guidelines.
2. All samples sent for testing must be accompanied by a requisition. A separate requisition must accompany each sample/test type
3. Avoid contamination with indigenous flora, while collecting samples. Clean collection site with disinfectant as per standard guidelines.
3. Collect all culture specimens prior to administration of any antimicrobial agents, where possible. If the patient is already on antibiotics, collect samples for culture just before next dose.

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4. Swabs are convenient but inferior to tissue and fluid for smear examinations and cultures. Tissue and fluid are essential for fungal and mycobacterial cultures.

5.1 Information on test requisition form

Requisition for all the tests should be made on e-hospital for digital TRF. For samples where registration on e-hospital cannot be done, manual registration should be done. In such cases samples must be accompanied by test requisitions, which must have the following information at a minimum

- Patient's full name
- Age & sex
- Ward/Bed/Unit
- Sample Type
- Date of collection
- Test (s) requested
- The name & signature of the requesting clinician

Desirable details include clinical diagnosis and other relevant information that may be required for interpretation of the laboratory findings.

5.2 Type & amount of Primary samples to be collected

Type of samples needed for various tests is as per table in Annexure 1.

For IPD patients, sample collection should be performed only by trained personnel. Instructions for patient self-collected samples must be given clearly. Sample information sheets for urine, stool and sputum are appended with this manual.

For OPD patients, instructions for self collection of samples like mid-stream urine, sputum and stool, will be provided to patients at the Microbiology OPD counter. Other samples will be collected by trained technicians' in the OPD Laboratory section.

5.3 Labeling Samples

The following essential information **must** be documented in a legible manner on the samples sent for testing from the ward/ collected in the OPD

- Patient identifiers- Name, Age, Sex.
- Sample Type
- Date of collection

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➤ Test(s) Requested

It is the responsibility of the person collecting and labeling the samples to match the identity of the patient as per requisition form.

Additional labeling will be made at the sample receiving counters.

5.4 Sample handling & transportation to the Department of Microbiology

Sample from the all Wards/OTs of the hospital must be transported as soon as possible and always within 4 hours of collection to the laboratory. In case unavoidable delays are anticipated, samples should be refrigerated, except for inoculated blood culture bottles and CSF samples, which must be placed in an incubator at 37⁰C. Appropriate bio-safety measures must be implemented while transporting samples from the ward/OT's to the laboratory.

5.5 Sample Receiving:

All samples will be received at the respective microbiology counters as given above, during the timings mentioned. For sample receiving, barcodes are generated at e-hospital, which can be used for sample tracking.

When a sample is received, a reference id is provided to the patient and the patient is informed of the likely time of reporting. The reference id is useful for quickly retrieving reports.

5.6 Sample acceptance and rejection:

Correctly collected, labeled and transported samples will be accepted for testing.

If sample is too little and multiple tests are ordered, possible testing will be done and a request for further sample for remaining tests will be placed while receiving sample.

Sample Rejection Criteria:

General criteria for all samples

(Based on factors which could result in incorrect test results or breach of biosafety protocols)

1. Gross contamination of outer surface of sample
2. Leaking vials/sample containers
3. Incorrect or unclear labeling of sample/requisition i.e., sample identification not clear
4. Samples in incorrect vials (including syringes)/with incorrect anticoagulant for test
5. Incorrectly transported samples likely to result in sample deterioration – too much delay from time of collection, with no clear information on interim storage conditions

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Additional criteria for rejection of serum samples

1. Grossly hemolysed samples
2. Excessively lipemic samples
3. Visibly contaminated samples
4. Clots in anticoagulated samples

Additional criteria for rejection of extra-pulmonary samples for CBNAAT

1. Pus samples in cotton swabs
2. Blood samples

Additional Information:

Further questions may be referred to the Microbiology laboratory or Microbiology resident on duty. For tests under government schemes/ programs additional information can be obtained from the respective laboratories. Unresolved queries/complaints/feedback may kindly be referred to faculty members of the department.

6.0 Appendices

Appendix 1: List of available laboratory examination

Appendix 2: HIV Laboratory (KG/Micro/3.1)

Appendix 3: CD4 Laboratory (KG/Micro/3.1)

Appendix 4: Viral Load Laboratory (KG/Micro/3.1)

Appendix 5: TB Laboratory (KG/Micro/3.2)

7.0 References:

International Standard ISO 15189: 2012 entitled “Medical Laboratories Particular Requirements for Quality and Competency” (current version).

8.0 Validity Statement

This document is valid for two year from the date of commencement.

9.0 Documents & Records

NA

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Appendix-1

(List of Available Laboratory Examination)

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S.N	Test Name	Preferred Specimen	Specimen collection & Transport	Optimum sample
1	BACTERIOLOGY			
1a	SMEAR FOR EXAMINATION			
1	Conjunctival Smear Examination	Conjunctival Smear	sterile swab	1 Swab
2	Smear Examination for Diphtheria	Throat Swab	sterile swab	1 Swab
3	smear examination for Gonococcus	Vaginal Swab	sterile swab	1 Swab
4	smear examination for bacterial vaginosis	High Vaginal Swab	sterile swab	1 Swab
1b	DIRECT MICROSCOPY			
1	DFA for Chlamydia trachomatis cervical swab	cervical swab	sterile swab	1 Swab
2	Microscopy for Bacterial Vaginosis			
3	wet mount for Trichomonas vaginalis			
1c	CULTURE & SENSITIVITY			
1	Blood Culture & Sensitivity (Automated Aerobic & Anaerobic) With ID/AST (MIC)-Bacteria	Blood	inoculated blood culture bottle	2 sets
2	Blood Culture & Sensitivity (Aerobic Bacterial) Manual ID/AST	Blood	inoculated blood culture bottle	2 sets
3	CSF Culture & Sensitivity(Automated ID/AST) (Bacterial)	CSF	universal sterile container	1-3 ml
4	CSF Culture & Sensitivity(Aerobic Bacterial) Manual ID and AST	CSF	universal sterile container	1-3 ml
5	Pus Culture & Sensitivity (Automated ID/AST with MIC) (Bacterial)	Pus	universal sterile container	1-5 ml

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6	Pus Culture & Sensitivity(Aerobic Bacterial) Manual ID & AST	Pus	universal sterile container	1-5 ml
7	Pus Culture & Sensitivity (Anaerobic- Bacterial)	Pus	universal sterile container	1-5 ml
8	Urine Culture & Sensitivity (Automated ID/AST) (Bacterial)	Early Morning Urine	universal sterile container	2-40 ml
9	Urine Culture & Sensitivity (Aerobic - Bacterial)- Manual ID and AST	Early Morning Urine	universal sterile container	2-40 ml
10	Sputum Culture & Sensitivity (Automated ID/AST) (Bacterial)	Sputum Culture & Sensitivity (Automated ID/AST) (Bacterial)	sputum	universal sterile container
11	Throat swab Culture & Sensitivity (Aerobic - Bacterial) Manual	Throat Swab	sterile swab	1 swab
12	Throat swab Culture & Sensitivity (Automated ID/AST) (Bacterial)	Throat Swab	sterile swab	1 swab
13	BAL Culture & Sensitivity (Automated ID/AST) (Bacterial)	BAL	universal sterile container	2-10 ml
14	Stool Culture & Sensitivity (Aerobic - Bacterial) Manual ID and AST	Stool	universal sterile container	2-40 ml of liquid/1 gm of semisolid
15	Stool Culture & Sensitivity (Automated ID/AST) (Bacterial)	Stool	universal sterile container	
16	Vaginal Swab Culture & Sensitivity(Automated ID/AST) (Bacterial)	Vaginal Swab	sterile swab	1 swab
17	Trichomonas vaginalis Culture & Sensitivity			
18	Gonococcus Bacterial Culture & Sensitivity	Vaginal Swab	sterile swab	1 swab
19	Body fluids Culture & Sensitivity(Automated	Body Fluid	universal sterile container	2- 10 ml

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	ID/AST) (Bacterial)			
20	CVP line Culture & Sensitivity (Aerobic)	CVP	tip of venous catheter in universal sterile container	5 cm of length
21	Tissue Culture & Sensitivity (Automated ID/AST) (Bacterial)	Tissue	universal sterile container	visible tissue
1d	BACTERIAL SEROLOGY			
1	Widal Test	Serum	plain vials	3 ml
2	RPR (Rapid Plasma Reagin) Test	Serum	plain vials	3 ml
3	V.D.R.L. Test	Serum	plain vials	3 ml
4	TPHA	Serum	plain vials	3 ml
5	Anti Brucella IgM antibodies	Serum	plain vials	3 ml
6	Anti Brucella IgG antibodies	Serum	plain vials	3 ml
7	Anti- Scrub typhus IgM by ELISA	Serum	plain vials	3 ml
8	Pertussis IgG ELISA	Serum	plain vials	3 ml
9	Anti Leptospira IgM by ELISA	Serum	plain vials	3 ml
1e	BACTERIAL IMMUNOLOGY & BIOMARKERS			
1	A.S.O. Titre	Serum	plain vials	3 ml
2	C- Reactive Protein (Quantitative)	Serum	plain vials	3 ml
3	C- Reactive Protein (Qualitative)	Serum	plain vials	3 ml
4	Procalcitonin (Rapid)	Serum	plain vials	3 ml
5	Procalcitonin (Quantitative)	Serum	plain vials	3 ml
6	Rheumatoid Factor (Latex Agglutination)	Serum	plain vials	3 ml
2	PARASITOLOGY			
2a	MICROSCOPY			
1	Microscopy FOR OVA & CYST (Routine)	Stool	universal sterile container	2-40 ml of liquid/1 gm of semisolid

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2	Microscopy for opportunistic parasite	Stool/Others	universal sterile container	2-40 ml of liquid/1 gm of semisolid/3ml
3	Microscopy for Malarial Parasite	Blood	plain vials	3 ml
4	Microscopy for Microfilaria	Blood	plain vials	3 ml
5	Microscopy for Pneumocystis carinii	BAL/Sputum	universal sterile container	2-5 ml
2b	PARASITIC SEROLOGY			
1	Rapid Malaria Test (HRP-2 based)	Blood/Serum	plain vials	3 ml
2	Rapid Malaria Test (p-LDH based)	Blood/Serum	plain vials	3 ml
3	Filaria antigen (Rapid)	Blood/Serum	plain vials	3 ml
4	Echinococcus IgG ELISA	Blood/Serum	plain vials	3 ml
5	Entamoeba histolytica antigen ELISA	Blood/Serum	plain vials	3 ml
6	Entamoeba histolytica IgM ELISA	Blood/Serum	plain vials	3 ml
7	Entamoeba histolytica IgG ELISA	Blood/Serum	plain vials	3 ml
8	Cysticercosis IgG ELISA	Blood/Serum	plain vials	3 ml
9	Cryptosporidium stool antigen ELISA	Blood/Serum	plain vials	3 ml
10	Leishmania antibody (Rapid)	Blood/Serum	plain vials	3 ml
11	Stool Antigen for Giardia (ELISA)	Blood/Serum	plain vials	3 ml
12	Toxoplasma IgM by ELISA	Blood/Serum	plain vials	3 ml
2c	RAPID TEST			
1	Stool for occult blood	Stool	universal sterile container	2-40 ml of liquid/1 gm of semisolid
3	MYCOLOGY			
3a	FUNGAL MICROSCOPY			
1	Smear for Fungus (KOH Mount)	Skin/Nail/Hair Scraping	sterile petri plate/black	visible sample

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			paper	
2	Immunofluorescence for Pneumocystis	Sputum	universal sterile container	2-5 ml
3	Smear examination for Candida	Vaginal Swab	sterile swab	1 swab
3b	FUNGAL CULTURE & SENSITIVITY			
1	Skin/Nail for C/S	Skin/Nail Scraping	sterile petri plate/black paper	visible sample
2	Pus for C/S	Pus	universal sterile container	1-5 ml
3	Sputum for C/S	Sputum	universal sterile container	2-5 ml
4	Throat swab (Automated) With ID/AST (MIC)-Yeast (Fungal)	Throat Swab	sterile swab	1 swab
5	BAL for Fungal C/S	BAL	universal sterile container	20-50 ml
6	CSF C/S (Automated) With ID/AST (MIC)-Yeast (Fungal)	CSF	universal sterile container	1-3 ml
7	Blood Culture for Filamentous Fungi (Manual)	Blood	biphasic blood culture bottle	1-4 ml pediatrics/ 8-10 ml adults
8	Blood Culture (Automated) With ID/AST (MIC)-Yeast (Fungal)	Blood	bactec bottle	1-4 ml pediatrics/ 8-10 ml adults
9	Body Fluids for C/S	Pleural/Pericardial	universal sterile container	20-50 ml/2-5 ml
10	Body fluids C/S (Automated ID/AST) Fungal	Pleural/Pericardial	universal sterile container	20-50 ml/2-5 ml
11	Urine C/S (Automated ID/AST) Fungal	Early Morning Urine	universal sterile container	20-50 ml
12	Urine for C/S	Early Morning Urine	universal sterile container	20-50 ml
13	Vaginal swab for C/S	Vaginal Swab	sterile swab	1 swab
14	Stool for C/S	Stool	universal sterile container	2-40 ml of liquid/1 gm of semisolid

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3c	FUNGAL SEROLOGY			
1	Cryptococcal antigen lateral flow (Rapid)	Blood/Serum	plain vials	1-5 ml
2	Galactomannan ELISA	Blood/Serum	plain vials	1-5 ml
4	TB LABORATORY			
1	AFB Microscopy (ZN Stain)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
2	AFB Microscopy (Auramin O Stain)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
3	AFB Culture - Solid (Manual)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
4	AFB Culture - Liquid (Automated)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
5	Identification (AFB culture)	AFB culture	Culture tube/ cryovial	--
6	AFB Culture and DST-First line drugs (Solid/Manual)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
7	AFB Culture and DST-First line drugs (Liquid/Automated)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
8	AFB Culture and DST-Second line drugs (Liquid/Automated)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
8	Line Probe Assay (Identification and susceptibility testing)	AFB Positive Sputum Sample; TB Culture Isolate	universal sterile screw capped container	2-5 ml
9	Gene Xpert/ CBNAAT (Identification and susceptibility testing)	Any Sample Other Than Blood	universal sterile screw capped container	2-5 ml
10	Drug susceptibility testing (Manual-Solid)	AFB culture	culture tube/ cryovial	--
11	Drug susceptibility testing (Automated-Liquid)	AFB culture	Culture tube/ cryovial	--
12	Smear For Lepra Bacilli	Skin Scraping/Nasal Swab	sterile slide/ swab	visible sample/1 swab

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5	ICTC			
1	Anti-HIV antibodies	Blood/Serum	plain vials	3 ml
2	CD4 count	Blood/Serum	plain vials	3 ml
3	HIV-1 Viral Load	Plasma	K2 EDTA Vials	6 ml
6	VIROLOGY			
6a	VIRAL SEROLOGY			
1	HBsAg ELISA	Blood/Serum	plain vials	3 ml
2	HBeAg ELISA	Blood/Serum	plain vials	3 ml
3	Anti- Hepatitis B Core Antigen IgM (Anti-HBc IgM) ELISA	Blood/Serum	plain vials	3 ml
4	Anti-Hepatitis B Surface Antigen (Anti-HbsAg)	Blood/Serum	plain vials	3 ml
5	HBeAb ELISA	Blood/Serum	plain vials	3 ml
6	Anti HBs Antibody ELISA	Blood/Serum	plain vials	3 ml
7	Anti- Hepatitis A Virus IgM Antibodies (anti-HAV IgM) (ELISA)	Blood/Serum	plain vials	3 ml
8	Anti- Hepatitis E Virus IgM Antibodies (anti-HEV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
9	Anti- Hepatitis D Virus IgM Antibodies (anti-HDV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
10	Anti HCV Total antibodies by ELISA	Blood/Serum	plain vials	3 ml
11	Anti- Cytomegalovirus IgM Antibodies (Anti-CMV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
12	Anti- Cytomegalovirus IgG Antibodies (Anti-CMV IgG)(ELISA)	Blood/Serum	plain vials	3 ml
13	CMV IgM ELISA	Blood/Serum	plain vials	3 ml
14	TORCH (IgM ELISA)	Blood/Serum	plain vials	3 ml
15	Anti-Chikungunya Virus IgM Antibodies (Anti-ChikV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
16	Anti-Herpes Simplex IgM Antibodies (Anti-HSV IgM)(ELISA)	Blood/Serum	plain vials	3 ml

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17	Anti-Measles Virus IgM Antibodies (Anti-Measles IgM)(ELISA)	Blood/Serum	plain vials	3 ml
18	Anti-Measles Virus IgG Antibodies (Anti-Measles IgG)(ELISA)	Blood/Serum	plain vials	3 ml
19	Anti-Mumps Virus IgM Antibodies (Anti-Mumps IgM)(ELISA)	Blood/Serum	plain vials	3 ml
20	Anti-Mumps Virus IgG Antibodies (Anti-Mumps IgG)(ELISA)	Blood/Serum	plain vials	3 ml
21	Anti-Varicella Zoster Virus IgM Antibodies (Anti-VZV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
22	Anti-Varicella Zoster Virus IgG Antibodies (Anti-VZV IgG)(ELISA)	Blood/Serum	plain vials	3 ml
23	Anti-Human Parvovirus B19 IgM Antibodies (Anti-B19V IgM)(ELISA)	Blood/Serum	plain vials	3 ml
24	Anti-Human Parvovirus B19 IgG Antibodies (Anti-B19V IgG)(ELISA)	Blood/Serum	plain vials	3 ml
25	Anti- Dengue Virus IgM	Blood/Serum	plain vials	3 ml
26	Dengue Virus NS1Ag	Blood/Serum	plain vials	3 ml
27	Anti- Japanese Encephalitis Virus IgM	Blood/Serum	plain vials	3 ml
28	Anti- West Nile Virus IgM Antibodies (Anti-WNV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
29	Anti-Epstein Barr Virus IgM Antibodies (Anti-EBV IgM)(ELISA)	Blood/Serum	plain vials	3 ml
30	Anti-Rubella Virus IgM Antibodies (Anti Rubella IgM)(ELISA)	Blood/Serum	plain vials	3 ml
31	Rotavirus Antigen in stool by ELISA	Blood/Serum	plain vials	3 ml
6b	MOLECULAR BIOLOGY			

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1	Real Time PCR For Hepatitis B Virus (Qualitative)	Blood/Serum	plain vials	3 ml
2	Real Time PCR with Viral Load estimation For Hepatitis B Virus	Blood/Serum	plain vials	3 ml
3	Real Time PCR For Hepatitis B Virus + Hepatitis C Virus (Quantitative)	Blood/Serum	plain vials	3 ml
4	Genotype For Hepatitis B Virus	Blood/Serum	plain vials	3 ml
5	Genotype For Hepatitis C Virus	Blood/Serum	plain vials	3 ml
6	Real Time PCR For Hepatitis C Virus (Qualitative)	Blood/Serum	plain vials	3 ml
7	Real Time PCR with viral load estimation For Hepatitis C Virus	Blood/Serum	plain vials	3 ml
8	Real Time PCR For Influenza A Virus	Blood/Serum	plain vials	3 ml
9	Real Time PCR For Influenza A Virus (subtype H1N1)	Blood/Serum	plain vials	3 ml
10	Real Time PCR For Influenza A Virus (subtype H3N2)	Blood/Serum	plain vials	3 ml
11	Real Time PCR For Influenza B Virus	Blood/Serum/CSF	plain vials	3 ml
12	Real Time PCR For Respiratory Syncytial Virus	Blood/Serum	plain vials	3 ml
13	Real Time PCR For Parainfluenza Virus 1,2,3,4	Blood/Serum	plain vials	3 ml
14	Real Time PCR For Herpes Simplex 1 Virus	Blood/Serum	plain vials	3 ml
15	Real Time PCR For Herpes Simplex 2 Virus	Blood/Serum	plain vials	3 ml
16	Real Time PCR For Varicella Zoster Virus	Blood/Serum	plain vials	3 ml

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17	Real Time PCR For Human Metapneumovirus	Nasal /Throat Swab	VTM	3 ml
18	Real Time PCR For Japanese Encephalitis Virus	Nasal /Throat Swab	VTM	3 ml
19	Real Time PCR For Dengue Virus	Nasal /Throat Swab	VTM	3 ml
20	Real Time PCR For Measles Virus	Nasal /Throat Swab	VTM	3 ml
21	Real Time PCR For Bocavirus	Nasal /Throat Swab	VTM	3 ml
22	Real Time PCR For Human Adenovirus	Nasal /Throat Swab	VTM	3 ml
23	Real Time PCR For Human Parvovirus B19	Nasal /Throat Swab	VTM	3 ml
24	Real Time PCR For Enterovirus	Nasal /Throat Swab	VTM	3 ml
25	Conventional PCR For Cytomegalovirus	Nasal /Throat Swab	VTM	3 ml
26	Conventional PCR For Rotavirus	Nasal /Throat Swab	VTM	3 ml
27	Conventional PCR For Norovirus	Blood/Serum	universal sterile container	5-15 ml
28	Conventional PCR For Astrovirus	Blood/Serum	universal sterile container	5-15 ml
29	Real Time PCR for Scrub typhus	Blood/Serum	universal sterile container	5-15 ml

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Appendix-2

(HIV Laboratory)

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GENERAL INFORMATION: HIV LABORATORY

- A. For HIV testing blood/serum samples collected from the wards/hospital (Indoor) or from the OPD or from the directly walk in clients should come to the HIV laboratory section of Department of Microbiology with the requisition form.
- B. As per the NACO guidelines patients first attend the HIV counseling provided by ICTC counselors in counseling room no. 116. Before HIV testing counselor's do the pre-test HIV counseling, take informed consent from patients, provide a unique PID no. after getting required information like name, age, sex, aadhar no., address and contact details and send them for sample collection at sample collection counter.
- C. For samples directly sent for HIV testing from IPD patients, it is expected that referring clinician has obtained the informed consent. In case of any doubt/problem clinicians can directly contact to HIV counselors. In special cases counselors may go to the bed side of admitted patients for counseling.
- D. After counseling patient go for registration at sample reception/ billing counter. The samples from the all Wards/OTs of the hospital must be transported in vacutainers/ sample transport vials and NOT IN SYRINGES.
- E. a) The test requisition form is raised on e-hospital using patient UHID, admission no. etc. After registering test orders in e-hospital, a zero bill receipt is generated for HIV tests. A barcode is then printed in 4 copies with UHID, sample number and name of patient (one for sample bill receipt, two for PID card and one for sample vial).
- b) If manual requisition form is raised the following essential information **must** be present in a legible manner on the form and on the samples sent for testing from the ward/ collected in the OPD
- Patient identifiers- Name, Age, Sex.
 - Sample Type
 - Date of collection
 - Test(s) Requested

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- F. It is the responsibility of the person collecting and labeling the samples to match the identity of the patient as per requisition form. Additional labeling will be made at the sample receiving counters.
- G. In sample collection area trained technician/phlebotomist collect the 3-5 ml blood by venipuncture in **red capped sterile vacutainer**.
- H. After collection samples are transported in sample transport box to the HIV laboratory section for HIV testing.
- I. In the laboratory, serum samples are separated by centrifugation of blood samples at 1500 rpm for 15-30 minutes in the sample separation room. After centrifugation separated serum are transferred with the help of a transfer pipette/ micropipette into labeled sterile leak proof specimen vial/ Eppendorf tube.
- J. Samples are then tested in the laboratory as per laboratory testing protocol following NACO strategy III. HIV Rapid Tests based on three different principles are used to give the confirmed HIV positive results.

Negative reports are given to those patients who are tested **non-reactive** on HIV rapid test I.

Positive reports are given to those patients who are tested **reactive on all the three HIV Rapid tests** (HIV Rapid Tests I, II & III).

If the sample is found **reactive on HIV rapid test I but either of two Rapid tests are found non-reactive**, it is **indeterminate sample** (if any risk behavior found) & no report issued to patient.

A fresh sample will be requested from patients after two week to confirm the HIV status of the patient. If upon re-testing of these samples same result is obtained, the sample will be sent to higher lab (SRL/NRL) for further confirmation and it will take around four weeks to get confirmed results.

If the sample is found **reactive on HIV rapid test I but either of two Rapid tests are found non-reactive, it is negative sample** if no risk behavior found & negative report issue to the patient.

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K. After testing reports are prepared by laboratory technician and after verification and signature of authorized signatory results of HIV tests are given to concerned person by counselors after post test counseling.

*Note: Where multiple samples have to be collected follow order of draw as in display chart (Addendum)

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Appendix-3

(CD4 Laboratory)

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CD4 LABORATORY

- A. For CD4 testing blood samples collected from ART plus center, Medicine OPD, KGMU, Lucknow
- B. As per the NACO guidelines patients first attend the HIV counseling provided in ART counseling room no. 13. Before CD4 testing counselor do the CD4 counseling, provide a pre ART/ART number and note all the information like name, age/sex, address, contact number etc. in “Green ART Card” and send them for blood sample collection in room no. 13.
- C. In the sample collection room blood is collected in EDTA vacutainer and an ID is allotted to patient which is also noted on green ART card and on sample vacutainer.
- D. All the blood samples are transported in EDTA vacutainers along with requisition form to CD4 laboratory section in cold chain.
- E. The following essential information must be present in legible manner on the requisition form
- Patient ID
 - Name
 - Age
 - Sex
 - Date of collection
 - Pre ART/ART No.
 - Old CD4 count
 - CD4 count
- F. It is responsibility of Laboratory technician collecting and labeling the sample to match the identity of the patient as per requisition form
- G. Sample testing for CD4 is done in CD4 laboratory section of Department of Microbiology, KGMU in Cy Flow counter

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- H. After testing the CD4 count are entered in requisition slip. After verification and signature of authorized signatory, reports issued to ART center.
- I. The CD4 results are also entered online in “IMS NACO”

*Note: Where multiple samples have to be collected follow order of draw as in display chart (Addendum)

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Appendix-4

(TB Laboratory)

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TUBERCULOSIS LABORATORY

1. Offered Tests and Selection of Right Tests

Test	Purpose	Eligible case (as per revised guideline- PMDT-2021)
AFB Examination (Auramine O / ZN Stain)	Diagnosis/ Treatment follow-up	✓ Presumptive-TB case ✓ Cases on-treatment for anti-tubercular drug
CBNAAT* (GeneXpert MTB/RIF)	Diagnosis	✓ Presumptive TB in key population (Extra-pulmonary case, Pediatric, PL-HIV or smear negative/NA with X-ray suggestive TB, contact of DR TB, other vulnerable groups, Non Responders etc.)
LPA (First Line)* (Genotype MTBDR_{plus})	Diagnosis	✓ Smear positive pulmonary TB cases, MTB-Rif Sensitive and or Resistant detected in CBNAAT
LPA (Second Line)* (Genotype MTBDR_{sl})	Diagnosis	✓ Smear positive pulmonary TB cases, MTB-Rif Resistant detected in CBNAAT
Culture* (MGIT 960 / LJ media)	Treatment follow-up / Diagnosis	✓ Cases on-treatment for anti-tubercular drug
Liquid Culture DST* (First Line)- MGIT 960	Diagnosis	✓ If drug resistant detected in LPA, LC DST for the drug Z would be subjected.
Liquid Culture DST* (Second Line)- MGIT 960	Diagnosis	✓ If drug resistant detected in LPA, LC DST for the drug Mfx, Lzd and Cfz, Bdq, Dlm (when available) would be subjected.
Solid Culture DST (First Line) (LJ media)	Diagnosis	✓ Blood Stained samples (that are in-appropriate to be tested by CBNAAT/LPA/ LC-DST).

* **Performed on a reflex testing manner** in compliance to "Integrated Diagnostic and Treatment Algorithm for Drug Resistant Tuberculosis" given in PMDT-Guidelines 2021. In case of any clarification regarding above table, PMDT Guidelines 2021 may be referred.

Note: Please avoid referring multiple/ irrational tests.

2. Sample Collection Procedure

- Refer the pulmonary TB suspects to DOT centre/ DMC at Department of Respiratory Medicine, KGMU, where a designated sample collection area and required materials (free of cost Falcon tube (50 ml centrifuge tube) /sample container and requisition form) are available.
- Suspect/patient/attendant may obtain the required materials from DOT centre/DMC.

2.1. Sputum (Expectorated): The early morning sputum is preferred. However on-spot collection of sputum is also acceptable but whenever possible efforts should be done to collect a second early morning sputum sample from such patients if the first one is negative.

- Label container with a unique patient identifier to match the requisition form. Make sure cap is on container tightly.
- Transport sample in a sealed and leak-proof box. Form should be kept away from specimen.

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2.2. Sputum (Induced): If the patient has difficulty producing a sputum specimen, then induction should be considered. Sputum production may be induced by the inhalation of a warm aerosol of sterile 5-10% sodium chloride in water produced by a nebulizer.

2.3. Gastric Lavage: This procedure can be employed where sputum production is unsuccessful. This technique requires professional attention and should only be attempted in the hospital. Gastric lavage is performed early in the morning before eating and at least 8 hours after the patient has eaten or taken oral drugs. 5-10mL specimen is required and must be neutralized with 100 mg of sodium carbonate.

2.4. Urine: An early morning midstream specimen should be collected. Send entire specimen. Multiple specimens over several days may be required to obtain a positive specimen. Urine sample is not appropriate for CBNNAT and LPA.

2.5. Fluids: Body fluids (spinal, pleural, pericardial, synovial, ascitic, pus, and bone marrow) must be aseptically collected and submitted in sterile containers. Keep refrigerated until transport.

2.6. Tissues: Any tissue to be cultured must be collected aseptically into sterile a container without fixatives or preservatives. If the specimen may dry, add sterile saline to keep moist. Do not place tissue specimen for culture into formalin. Keep refrigerated until transport.

2.7. Blood: NOT ACCEPTED

2.8. Sample Submission: Suspect/patient/attendant will submit the sputum samples (as mentioned in point No 2.1 and 2.2) at DMC (Department of Respiratory Medicine, KGMU). However, submission of other samples (as mentioned in point No 2.3 to 2.6) will be done as mentioned in Table No. 1.2 page No 5 of this manual along with properly/legibly/completely filled test request form (annexure 15A). Ensure sample order on e-hospital also. The registration of sample at Nikshay portal and generation of Nikshay ID is mandatory. This is the responsibility of respective clinician/Department/Health Facility.

3. Testing Fees: FREE of cost.

4. Sample Rejection Criteria:

- A) Sample in wrong container (syringes/swabs);
- B) Sample leakage/broken container;
- C) Insufficient volume of sample
- D) Specimen and requisition form differ
- E) Sample received > 72 hrs from it's collection
- F) Visibly contaminated specimen
- G) Blood stained/urine samples for CBNAAT/LPA)
- H) Specimen sent without Test request ID in Nikshay

5. Usual turn-around time:

A) AFB Microscopy: 1 Day

B) CBNAAT: 3 Day

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C) LPA: 5 Days

D) Liquid Culture: 10-45 Days

E) Liquid Culture DST: 50 Days

F) Solid Culture: 30-60 Days

G) Solid Culture DST: 85 Days

Note: TAT may exceed in conditions like sample load beyond the routine processing capacity of Lab, repeat testing requirement.

6.0. Contact for any help:

Room No. 116, New OPD Building KGMU, Chowk, Lucknow

Email: irluplno@rnctp.org

Dr. Urmila Singh (Mob: 9936057067); Dr. Vijay Kumar (Mob: 8126623166)

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Appendix-5

(Viral Load Laboratory)

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VIRAL LOAD LABORATORY

1. Sample Collection Procedure

- A. All registered individuals on ART who are scheduled for VL testing is referred by the Medical Officer to the technician at the ARTC for sample collection with filled in triplicate carbon copies of the Test Requisition and Result Form (TRRF).
- B. On receiving the patient, the laboratory technician verifies the TRRF; confirm the identity of the patient by Unique ART Number and at least one other identifier such as name, age, gender etc.
- C. Unique VL test ID (17 digit) is generated by the laboratory technician at the ART centre at the time of blood collection.
- D. Prior to the collection of the sample by Venipuncture, explain the procedure to the patient.
- E. Labelled the blood collection tube with 17 digit number/ any of two identifiers and the date and time of collection using cryo labels.
- F. Strictly follow the standard precautions and collect the blood sample by wearing powder free gloves.
- G. Collect 6 ml of whole blood sample in a K2 EDTA evacuated tube using an eclipse needle (usually 23G) while 3 ml blood should be drawn from infants less than one year.
- H. In children and adults with thin fragile veins where it may be difficult to draw blood with vacutainer alternative blood collection devices to be used.
- I. The EDTA tubes should be gently inverted 8-10 times to ensure proper mixing of whole blood and EDTA to prevent clotting. The tubes are kept upright at room temperature (15-30 C) before samples are transported to the testing lab.
- J. Following sample collection the date and time of sample collection must be entered in the TRRF. This information will be also entered manually and digitally respectively in the register and IMS. Completed TRRF in triplicate is accompanying the sample throughout handling and transport.
- K. Transport the samples to the receiving laboratory by hand delivery by a trained person.

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- L. All samples must be transported to VL lab within 5 hrs. of collection so that plasma can be separated within 6 hrs. of sample collection at VL Lab.
- M. Centrifuge the sample tubes at 2000-2500 rpm for 10-15 minutes. Suitable plasma samples are clear and have a slight yellow tint with defined buffy coat and red blood cell layers.
- N. Following centrifugation transfer maximum amount of clear straw colored plasma in two 1.8 ml vials and put ART no. as well as bar code no. on every vial.

2. Sample Storage

- A. Store the samples in -20°C till the viral load testing.
- B. Post testing, the samples are to be stored at -70 C or lower, to preserve RNA, for 1 year.
- C. Once plasma is frozen, it must be always transported frozen to avoid freeze thaw cycles.

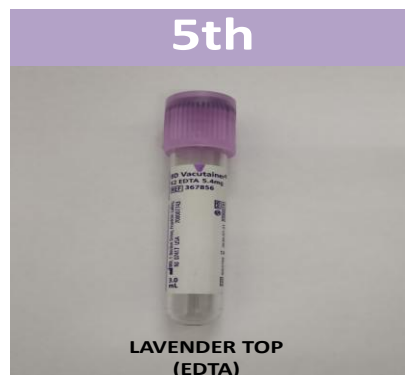
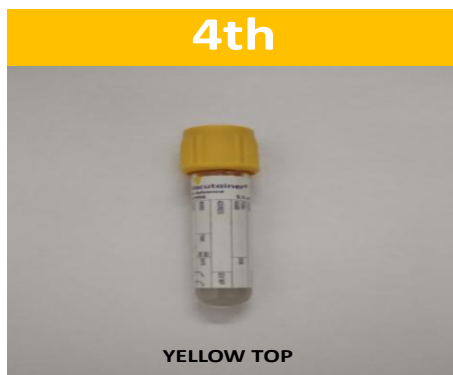
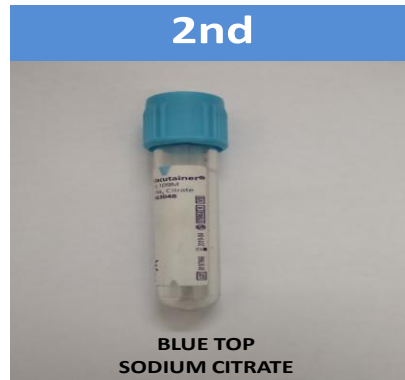
3. Acceptance / Rejection Criteria For Samples

Acceptance Criteria	Rejection Criteria
<ul style="list-style-type: none">• Sample properly labeled• Sample tube integrity maintained, no leakage• Sample label matched with TRF• Adequate volume• Clear plasma• Transport temperature between 2-8 degree C	<ul style="list-style-type: none">• Hemolysed samples• Grossly lipemic samples• Contaminated samples• Inadequate volume• Leaking tubes• Improperly label samples• Temperature deviation• Samples from HIV-2 infected individuals
In case of any discrepancy observed or rejection of samples: Viral load lab to inform the ARTC immediately by phone or email	

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BLOOD SPECIMEN COLLECTION ORDER OF DRAW



Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw is:

1. **First-** blood culture bottle (**BD BacT/ALERT**)
2. ***Second-** coagulation tube (**light blue top**).
3. ***Third** – clot activator serum tube (**red top**)
4. **Last draw** - additive tubes in this order:
 1. SST (**gold top**). Contains a gel separator and clot activator.
 2. EDTA (**lavender top**)
 3. Oxalate/fluoride (**light gray top**)

Examine list of tests ordered.

Decide on tubes to be used for appropriate sample collection.

Lineup tubes using chart above for order of draw. Proceed with sample collection after skin cleaning as per SOP.

Note: *Order 2nd and 3rd to be interchanged if non additive serum collection tube used.

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Issue No	Effective date	Revised By	Revision Summary
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Document Reading List

In the matrix below the documents are indicated which should be read and understood by the staff members of Department of Microbiology, KGMU. Write your signature in the appropriate cell in the row where the document is indicated that you have read.

The following employees have read and understood the Doc.- KG/Micro/03

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