Release Authorization

This Primary Specimen Manual

(KEM / Micro /TP 1 / PSM)

is released under the authority of

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Seth G. S. Medical College & K. E. M. Hospital

and is the property of

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***HOD – Head of Department**

All heads of departments are requested to circulate this primary specimen manual to all the staff members and make this available in the wards.

This information is also available on KEM intranet.

AMENDMENT RECORD

Sr No	Pg No	Section/ Clause / Para / Line	Date of Amendment	Amendment made	Reason for amendment	Signature of person authorizing amendment
1	2	Distribution list	3.1.2022	Online availability No physical print copies	Ease of use and larger access	
	15-18	Contact person of various divisions	3.1.2022	Transfer of faculty and reassignment of divisions	Transfer of faculty	
	26	Test 27 SARS-CoV-2 Antibody test	3.1.2022	Addition in limitation column – All positive results to be correlated clinically.	Completeness	
	73	TAT SARS-CoV-2 IgG antibody	3.1.2022	TAT for SARS-CoV-2 IgG antibody added	Inclusion	

List of Abbreviations

Abbreviations	Full Form
Ab	Antibody
AFST	Antifungal Susceptibility Test
ART	Anti-Retroviral Therapy
CBWTF	Common Biomedical Waste Treatment Facility
COVID	Corona Virus Disease
CRBSI	Catheter Related Blood Stream Infection
CVTS	Cardiovascular and Thoracic Surgery
DST	Drug Susceptibility test
ENT	Ear, Nose and Throat
EPTB	Extra-pulmonary Tuberculosis
GAS	Group A Streptococci
GI	Gastrointestinal
HOD	Head of Department
hrs	Hours
ICTC	Integrated Counselling and Testing Centre
ILI	Influenza like illness
MIC	Minimum Inhibitory Concentration
OBGY	Obstetrics and Gynaecology
Ped	Paediatric
PSM	Preventive and Social Medicine
PTB	Pulmonary Tuberculosis
MSB	Multi-storeyed Building
NTEP	National Tuberculosis Elimination Programme
RF	Rheumatoid factor
RDT	Rapid Diagnostic Test
ASO	Anti Streptolysin O
ELISA	Enzyme Linked Immunosorbent Assay
RAT	Rapid Antigen Test (COVID-19)
RDT	Rapid Diagnostic Test

RT-PCR	Reverse transcriptase Polymerase Chain Reaction
RPR	Rapid Plasma Reagin
SRF	Specimen Referral Form
V.D.R.L	Venereal Disease Research Laboratory
VRDL	Viral Research and Diagnostic Laboratory
PPE	Personal Protective Equipment
TAT	Turnaround time
TT	Tetanus toxoid
WGS	Whole Genome Sequencing

Contents

1. FOREWORD

This Primary Specimen Manual has been prepared to provide an overview of the tests offered, their indications and limitations and also facilitate the process of aseptic and standardized collection and transportation of clinical specimens for microbiological investigations. This 7th issue has incorporated COVID-19 RT-PCR, RAT and antibody tests. Recipients of this manual are requested to share this manual with all members of the department which includes interns, residents, registrars, nursing staff and teaching faculty.

2. INTRODUCTION

'The result of a test is only as good as the quality of the specimen.' A good quality specimen is an important pre-analytic criterion for the accuracy of a test result. This manual is intended to provide the clinicians and the laboratory personnel alike, the instructions on what constitutes appropriate specimens, and where and how they need to be sent / transported.

The Department of Microbiology offers diagnostic services for infectious diseases through its different divisions viz. Clinical Bacteriology, Molecular Diagnostics, Mycobacteriology, Mycology, Parasitology, Serology, and Virology & Immunology including ICTC. Apart from these divisions, the department also offers emergency laboratory services after routine hours for processing specimens of emergency nature or from seriously ill patients. The records of specimens processed are maintained without affecting patient confidentiality by restricting access of these records to only laboratory staff.

All health care workers should complete the full course of Hepatitis B vaccination and also receive TT. HCWs are also requested to complete their COVID-19 vaccination series as and when rolled out.

QUALITY ASSURANCE

Services are provided using approved reagents and kits, calibrated equipment and controls, and trained and proficient manpower authorized by qualified microbiologists. External Quality Assessment and continual improvement programs are in place to assure the quality of the results generated. The laboratory is approved by ICMR for COVID-19 related RT-PCR test.

SCOPE

This manual is meant for all those health care workers who are involved with specimen collection, labeling, transport, storage, handling and disposal.

PURPOSE

The purpose of this manual is to facilitate collection and transport of appropriate specimens in a manner that reduces the risk of exposure to blood and body fluids, maintains confidentiality as required and complies with standard collection protocols.

RESPONSIBILITY

a) Health care workers

- Should follow the recommendations / procedures described in this manual
- In case a clarification is required, should contact the division in charge or head of the department (Section 5)
- Should follow standard precautions while collecting, handling and transporting specimens (Section 3)
- Ensure that appropriate specimen is collected in adequate quantity in appropriate containers which are labelled and transported along with an appropriately filled requisition form immediately to the laboratory
- Biohazard spill should be attended to immediately (section 25)
- In the event of a needle stick injury, immediate action as per the protocol is indicated (Section 24)

b) Hospital administration

- Provide the containers and PPE as required for collection and transport
- Facilitate immunization of health care workers

c) Head of Laboratory

- Sensitise health care workers on procedures described in the manual through designated staff
- Make a copy of the manual available to all the departments

d) Microbiology Supervisory Staff and Division in charge

- Periodically audit compliance and suitability of the procedures
- Take corrective action in case non-compliance is detected

3. STANDARD PRECAUTIONS (collection, handling, transport)

These precautions should be followed by all health care workers to prevent the transmission of infectious agents while providing health care which also includes specimen collection, handling and transport.

- All clinical specimens should be considered as potentially infectious.
- All cuts and dressings should be completely covered with impervious dressing.
- Appropriate personal protective equipment should be worn while performing collection as per expected exposure risk (e.g. a pair of clean gloves).
- Hands should be washed before and after a procedure irrespective of glove use.

- Where there is a risk of splash occurring, face shield and gown should be worn in addition.
- Follow safe injection practices. Wear a surgical mask when performing lumbar punctures.
- N95 respirators are recommended while collecting throat swabs from patients with infections that are transmitted by droplets such as suspected flu, diphtheria, COVID-19 etc.
- N95 respirators are recommended to be worn while collecting specimen using a bronchoscope from patients with infections that are transmitted by droplet nuclei such as flu, tuberculosis,COVID-19.
- All spills of blood and body fluids should be decontaminated with an absorbent containing 0.5-1% sodium hypochlorite (freshly prepared) immediately.(Refer pg 61)
- Used items must be discarded as per KEMH waste disposal policy.
- The advent of COVID-19 has necessitated greater emphasis on the rational use of personal protective equipment to prevent the transmission of disease. Use of N95 masks / respirators is mandatory while providing clinical care in COVID related areas, in laboratory and while performing autopsies. Practicing appropriate donning and doffing of PPE is essential to prevent transmission to oneself and others.

4. LABORATORY WORKING HOURS

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

	Weekdays	9.00 a.m. to 4.00 p.m.

Routine working hours – All divisions	Saturdays & Bank Holidays	9.00 a.m. to 12.30 p.m.
Emergency laboratory Services	Weekdays	4.00 p.m. to next day 9.00 a.m.
	Saturdays / Bank Holidays	12.30 p.m. to Sunday / Next working day 9.00 a.m.
	Sundays / O.P.D Holidays	9.00 a.m. to Monday / Next working day 9.00 a.m.
COVID-19 RT-PCR and RAT	All days	Routine patient samples will be accepted till 5 pm. Samples of COVID symptomatic / critically ill asymptomatic / deceased patients will be accepted throughout the day.

SPECIMEN ACCEPTANCE TIMINGS:

	Division	Timing
OPD patients	All divisions	9.00 a.m. – 11.00 a.m.
Indoor patients	All divisions	9.00 a.m. – 12.00 p.m.
Blood / Body fluids / Aspirated pus/ Tissue / Ocular specimens / E.N.T specimens, Stool for cholera	Serology, Clinical Bacteriology, Mycology, Mycobacteriology and Parasitology	During the entire working period
Urine, Stool (other than for cholera) and Sputum	Clinical Bacteriology	9.00 a.m. – 11.00 a.m.
Direct walk in clients	Virology and Immunology / ICTC	9.00 a.m to 4.00 p.m
COVID-19 RT-PCR and RAT	Molecular Diagnosis	Routine patient samples will be accepted from 9.00 a.m till 5 pm. Samples of COVID symptomatic / critically ill

		asymptomatic / deceased patients will be accepted throughout the day.

5. TESTS / SERVICES OFFERED:

Division / Location	**Tests offered**	**Specimen type * and number where applicable**	**Contact Person with intercom number**
Clinical Bacteriology 7th floor, MSB	Microscopy& Culture for aerobic bacteria and anaerobic bacteria Antimicrobial susceptibility test on clinically relevant aerobic bacteria **MIC – Vitek2** Environmental sampling and sterility assurance tests as required	All specimens collected aseptically in sterile containers	Dr Lona Dash / Dr Gita Nataraj 7552 / 7527
	BACTEC Aerobic plus for adults (as per availability)	Blood	
	BACTEC Peds plus for children / neonates (as per availability)	Blood	
	MIC (as per availability Vitek 2 / E test strips)	On request	
Molecular Diagnosis 5th floor, MSB	HIV viral load for patients referred from ART	Whole blood in EDTA evacuated tube	Dr Shashir Wanjare / Dr. Ranjana Thate / Dr. Gita Nataraj 7039 / 7552
	HBV viral load (for patients referred from GI OPD)		
	HCV viral load (for patients referred from GI OPD)		

Division / Location	**Tests offered**	**Specimen type * and number where applicable**	**Contact Person with intercom number**
Molecular Diagnosis (7th floor)	COVID-19 RT-PCR	Nasopharyngeal swab + oropharyngeal swab OR Nasal swab + Throat swab OR Sputum in case of pneumonia	Dr. Priyanka Prasad / Dr. Gita Nataraj 7552
Mycology 5th floor, MSB	Microscopy , Culture, Identification for fungi, AST for yeasts	All specimens collected aseptically in sterile containers	Dr Vasant Baradkar / Dr Pallavi Surase 7857 / 7824
Mycobacteriology 5th floor, MSB	Microscopy (LED fluorescent microscopy)	Sputum** – at least 2 specimens of which one is early morning and the other is spot.	Dr Swapna Kanade 7827
		Gastric lavage – 3 specimens collected on 3 different days, Other specimens – One or more	
	Culture - MGIT	At least 3 ml in case of non-tissue specimens	Dr Swapna Kanade 7827

Division / Location	**Tests offered**	**Specimen type * and number where applicable**	**Contact Person with intercom number**
	1st Line, 2nd line and Pyrazinamide DST - MGIT	Sputum (currently for followup patients as per NTEP guidelines)	
	Xpert MTB/RIF ** assay# for simultaneous detection of MTB and Rif resistance as per programmatic recommendations	Sputum specimen x 2 / GL x 2-3 / Extra pulmonary in Falcon tube (procured from DOTS centre, 5th floor CVTS building)	
Parasitology 5th floor, MSB	Stool – Routine and Microscopy Stool and other body fluids / tissues –for potential and opportunist parasites	- Stool -BAL -Other respiratory specimens -Hydatid fluid -Other body fluids	Dr. Alpana Wagh / Dr. Vasant Baradkar 7857 / 7832
	RDT - malarial antigen	Whole blood / finger prick	
Serology 5th floor, MSB	ASO Dengue – NS1 antigen (Rapid / ELISA) Dengue – IgG and IgM antibodies (Rapid / ELISA) Leptospirosis – IgM Antibodies (Rapid / ELISA) RF Widal RPR / V.D.R.L	Whole blood collected in clean, dry, plain test tube / red top evacuated tubes.	Dr Vijaya Torane 7984 / 7985

Division / Location	**Tests offered**	**Specimen type * and number where applicable**	**Contact Person with intercom number**
	Chikungunya IgM Antibody **Covid-19 IgG antibody** **Referral of specimens to PCR laboratory** at Kasturba Hospital for Leptospirosis, Dengue pdmH1N1/2009	Serum PCR – 3-5 ml blood in purple cap (EDTA) evacuated tubes and transported in cold chain Collect sample (nasal/throat) using the nylon swab provided with VTM kit, place in VTM and transport in cold chain	
	COVID-19 RAT	As provided in the kit. Usually NP swab with buffer solution	Dr. Priyanka Prasad
Virology and Immunology 5th floor, MSB –	ICTC @ HIV – antibody detection HCV – antibody detection HBsAg detection RPR CD4 count enumeration	Whole blood collected in clean, dry, plain test tube / yellow or red evacuated tube EDTA evacuated tube	Dr. Shashir Wanjare / Dr Vaishali Surase/ Dr. Ranjana Thate 7039/7825

*Details about the specimen collection will be provided in the sections below.

**, # Specimens should be accompanied by appropriately filled RNTCP laboratory forms
@ Specimens should be accompanied by appropriately filled written informed consent form (Marathi / English) for HIV antibody test

- All sample containers should be adequately labelled.
- All samples should be accompanied by adequately filled requisition form.

6. TEST INDICATIONS AND LIMITATIONS

Sr.no.	Specimen / test performed	Indications (major)	Limitations
CLINICAL BACTERIOLOGY DIVISION			
1	**Blood culture (conventional)** Aerobic culture & Antimicrobial susceptibility test	CRBSI, Enteric fever, Infection of prosthetic material (implants), Infective endocarditis (IE), Meningitis, Osteomyelitis, Pneumonia, PUO, Septicemia	Usually positive only in acute phase. Multiple specimens required in IE. Lesser volumes (<10-20 ml) decrease yield. Blood culture contamination during collection can lead to pseudobacteremia.
2	**Blood culture (Automated method BACTEC** 9050) Rapid aerobic bacterial culture by automated system	Same as above If patient on antimicrobial, collect just before the next dose is due.	Pre-incubation of automated blood cultures reduces the yield of Pseudomonas, Streptococcus and Candida spp. In case of delay , store at room temperature (20-30°C)
3	**Normally sterile body fluids** – culture C.S.F, Pleural, Pericardial,	Infection at respective sites	Negative microscopy or culture does not rule out disease. Larger volumes improve sensitivity.

Sr.no.	Specimen / test performed	Indications (major)	Limitations
	Peritoneal (Ascitic), Joint, Smear, Culture and Antimicrobial susceptibility test		
4	**Throat swab from suspected diphtheria** case Smear examination by microscopy for Diphtheria Culture on appropriate media	Suspected diphtheria	Microscopy – unreliable A positive culture followed by demonstration of exotoxin production is the gold standard
5	**Sputum -** Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired	Both sensitivity and specificity are considered </= 50% unless expectorated sputum is purulent.
6	**Respiratory samples culture** (mini BAL, BAL, endotracheal aspirate) Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired Counts >/= 10^4 cfu/ml correlates better with disease though not always	Difficult to distinguish colonization from infection even with quantitative cultures. Clinical correlation essential.
7	**Miscellaneous** (Pharyngeal swabs, Skin scraping) Smear, Culture and Antimicrobial susceptibility test	Suspected streptococcal pharyngitis, Localised skin infections	Used to rule in disease. Collect samples in suspected GAS infection patients from posterior pharyngeal wall and tonsils. The isolate needs to be clinically correlated for its significance as a colonizer / pathogen.

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			Swabs need to be transported to lab immediately. A dried swab is detrimental to growth and can give false negative results.
8	**Ocular specimens** (conjunctival swab, Corneal scrapings, corneal button, eye discharge, vitreous humor, cornea) Smear, Culture and Antimicrobial susceptibility test	Conjunctivitis, corneal transplant, corneal ulcer , other eye infections trachoma,	Negative microscopy or culture does not rule out disease. Bedside inoculation on appropriate media improves yield provided aseptic practices are followed.
9	**Pus** Smear, Culture and Antimicrobial susceptibility test	Localised skin or organ specific	Sensitivity – 70% Specificity - High
10	**Wound swab** Smear examination by microscopy	Bacterial cellulitis, gas gangrene	Microscopy and culture unreliable. Collect tissue material or purulent discharge whenever possible.
11	**Tissue** (other appropriate specimen) for gas gangrene Smear and Culture (anaerobic)	Gas gangrene, local infection, intra-operative	Gas gangrene is a clinical diagnosis. Microscopy cannot characterize the genus. A negative test does not rule out disease. Swabs collected without appropriate debridement will yield contamination / false negative result.
12	**Specimens from female genital tract**	Vaginitis, cervicitis, urethritis	Specimens from lower genital tract will be contaminated

Sr.no.	Specimen / test performed	Indications (major)	Limitations
	(Vaginal /cervical swab, Urethral discharge, product of conception) and urethral discharge Smear, Culture and Antimicrobial susceptibility test		with normal flora and difficult to interpret.
13	**Stool Microscopy –** hanging drop	Diarrhoeas, purulent enterocolitis	A negative test for darting motility does not rule out cholera (sensitivity and specificity ~ 60%)
14	**Stool Culture & Antimicrobial susceptibility test**	Diarrhoeas, dysentery, purulent enterocolitis	Necessary to process specimens immediately to prevent overgrowth by normal flora.
15	**Urine Smear, culture & Antimicrobial susceptibility test**	Recurrent / Complicated UTI Known UTI with treatment failure PUO Asymptomatic bacteriuria in pregnant women	-False positives with clean catch urine specimens is high since the urine sample passes through the distal urethra and can become contaminated with commensal bacteria. -Culture of urine from urine collection bag gives false positive result. -Culture positive urine in a sick patient does not exclude another site of serious infection. -Prior antibiotic therapy may lead to negative urine culture in patients with UTI.

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			-Sterile pyuria may be due to causes other than non-fastidious aerobic bacteria.
SEROLOGY DIVISION			
16	**RF Test for rheumatoid factors**	In-vitro detection of Rheumatoid factor in patients serum by latex agglutination method.	-Does not provide definite diagnosis of rheumatoid arthritis and should always be correlated clinically -False positive results are seen in auto immune diseases, acute bacterial and viral diseases - Test can be negative in some patients with Rheumatoid arthritis.
17	**ASO test**	Detection of antibodies to streptolysin O produced by group A beta hemolytic streptococci by latex agglutination method.	-All positive results should always be correlated clinically -Nonspecific results are seen in lipemic, hemolysed, contaminated and high protein content serum -False positive results are seen with the use of plasma instead of serum
18	**RPR / VDRL Test**	For detection and quantification of reagin antibody in serum/plasma and spinal fluid in syphilitic patients.	-Nonspecific test for syphilis - All positive results should be correlated clinically

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			-All positive samples should be confirmed by TPHA or FTA ABS - False Negative: early primary syphilis; in secondary syphilis because of prozone reaction; and in some cases of late syphilis. -Biological false positive occurs in conditions such as - infectious mononucleosis, viral pneumonia, malaria, lepromatous leprosy, pregnancy, collagen disease, other autoimmune diseases
19	**Widal Test**	Detection of typhoid fever or paratyphoid fever by agglutination method.	-Not a specific (65%) or sensitive test (65%) -All reactive titres should be correlated clinically - TAB vaccinated patients may show high titres
20	**LeptoIgM rapid**	Qualitative detection of IgM class of Leptospira specific antibodies in human serum/ plasma/whole blood by rapid immunochromatography method.	- Less specific than ELISA -All positive results should always be correlated clinically -Intensity of test line depends on the stage of the disease and titre of the antibody

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			-Samples collected during early stage of disease (0-7days) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
21	**Lepto IgM ELISA**	Qualitative detection of IgM class of antibodies against Leptospira by ELISA method.	Same as above
22	**Dengue NS1 – Rapid (As per notification received from MoHFW, GoI, a positive result by rapid test will be considered probable due to its poor sensitivity and specificity)**	Qualitative detection of non-structural protein 1 (NS1) of dengue virus in serum/plasma by rapid immunochromatography method during first week of illness.	Samples collected during late stage of disease (after 7 - 9 days of fever) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
23	**Dengue NS1 – ELISA (In a clinically compatible case, demonstration of NS1 antigen by ELISA is considered confirmatory)**	Same as above	Same as above
24	**Dengue IgG/IgM Rapid (As per notification received from MoHFW, GoI, a positive result by**	Qualitative detection of IgG or IgM class of antibodies against dengue virus in human serum/ plasma by rapid	- Not as specific or sensitive as ELISA -All positive results should always be correlated clinically -Intensity of test line depends on the stage

Sr.no.	Specimen / test performed	Indications (major)	Limitations
	rapid test will be considered probable due to its poor sensitivity and specificity)	immunochromatography method	of the disease and titre of the antibody -Samples collected during early stage of disease (0-7days) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
25	**Dengue IgM ELISA (In a clinically compatible case, demonstration of Dengue IgM antibody by ELISA is considered confirmatory)**	Same as above	Same as above
26	**Chikungunya Antibody – ELISA (as per availability of kits)**	Qualitative detection of IgM class of antibodies against Chikungunya virus by ELISA method.	All positive results should be correlated clinically
27	**SARS CoV-2 IgG ELISA**	Qualitative detection of IgG class of antibodies against SARS CoV-2 virus by ELISA method.	The test could be positive in those who have had an exposure to the virus in the past and / or have been vaccinated. All positive results should be correlated clinically.
28	**COVID-19 RAT**	Symptomatic (cough, fever, sore throat, loss of taste and/or smell, breathlessness and/or	The sensitivity of the test being lower than RT-PCR, all RAT negative symptomatic individuals should be

Sr.no.	Specimen / test performed	Indications (major)	Limitations
		other respiratory symptoms) individuals. At-risk contacts of laboratory confirmed cases. [At-risk contacts are elderly (>60yr) and individuals with co-morbidity such as diabetes, hypertension, chronic lung or kidney disease, malignancy, obesity etc].	confirmed by RT-PCR. The test has to be processed within 30 minutes of collection.
MYCOBACTERIOLOGY DIVISION (Also refer to Appendix 9)			
29	**Microscopy**	Clinical suspicion of PTB / EPTB	Sensitivity low (10^5 orgs/ml)
30	**Culture**	All EPTB cases and suspected MDRTB cases as per recent PMDT guidelines	Solid culture – 4 / 6 weeks for report Liquid culture - contamination
31	**XpertMTB/RIF assay**	Initial diagnostic tests for MDRTB suspects, pediatric TB, all HIV positive TB suspects and all extrapulmonary TB	Detects rifampicin resistance only. Cannot predict for other anti-TB drugs other than INH.
PARASITOLOGY DIVISION			
32	**Stool / other specimens - Microscopy**	Suspected parasitic infection in immunocompetent / immunocompromised patients	For detecting trophozoites, fresh stool specimen essential to be examined within the hour of collection.

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			A negative result on a single stool specimen does not rule out parasitic presence.
33	**Blood – RDT malarial antigen**	Clinically suspected malaria cases	- Detection limit is usually 200 parasites / μl. May not detect low level parasitemia. -Use of RDT does not eliminate the need for malaria microscopy. -The currently approved RDT detects 2 different malaria antigens; one is specific for P. falciparum and the other is found in all 4 human species of malaria. Thus, microscopy is needed to determine the species of malaria other than *P.falciparum.*
MYCOLOGY DIVISION			
34	**Any specimen – Microscopy(KOH)**	Suspected superficial or deep fungal infection	-The sensitivity of a KOH prep is relatively low (20-75%) -The test may require overnight incubation for complete disintegration of thicker specimens like hair, nail, or biopsy

Sr.no.	Specimen / test performed	Indications (major)	Limitations
35	**Microscopy – India ink**	Suspected cryptococcal infection	-The diagnosis of *C. neoformans* by India ink staining should be considered a presumptive result - Culture, biochemical and serological testing is recommended for final identification. Some strains of *C. neoformans*, as well as other cryptococci may not produce discernible capsule
36	**Culture**	Suspected superficial or deep fungal infection	-Longer time required for growth of different fungi -Contamination by saprophytic fungi
VIROLOGY AND IMMUNOLOGY DIVISION			
37	**HIV Antibody tests (Rapid)**	-Patients who present with symptoms suggestive of HIV infection. Examples pneumonia, TB or persistent diarrhoea. -Patients with conditions that could be associated with HIV such as STI/RTI. -Prevention of parent (mother) to child	-False Negative result : in window period & terminal stage of HIV disease -False positive result: autoimmune disease, multiple blood transfusion, pregnancy etc.

Sr.no.	Specimen / test performed	Indications (major)	Limitations
		transmission - pregnant women who register at ANCs. These also include pregnant women who directly come in labour without any antenatal check-up	
38	**HBsAg ELISA**	• Signs/symptoms suggestive of hepatitis • H/o exposure	-False Negative : during incubation period -False positive: due to presence of other antigens or elevated levels of Rheumatoid factor
39	**Anti HCV ELISA**	• Signs/symptom suggestive of hepatitis • H/o exposure	-False Negative: in window period -False positive: elevated levels of Rheumatoid factor - Cannot differentiate recent from past infection
40	**RPR test**	• Direct walk in patients with high risk behavior • Patients referred by the STI counselor	-See page **22** above
41	**CD4 count**	• HIV positive patients referred from the ART centre	-Nonspecific marker which can be affected

Sr.no.	Specimen / test performed	Indications (major)	Limitations
			by many other conditions
MOLECULAR DIAGNOSIS			
42	**HIV viral load**	Monitoring response to treatment	The detection limit (sensitivity) varies between kits. The current test has a detection limit of 150 RNA copies / ml/
43	**HBV viral load**	Initiate treatment and monitor response to therapy	Limit of detection 6 IU/ml
44	**HCV viral load**		Limit of detection 9 IU/ml
45	**COVID-19 RT-PCR**	Diagnosis of COVID-19 . Testing strategy as per periodic ICMR advisory. Repeat testing for confirming non-infectiousness in severely ill hospitalised COVID 19 patients prior to discharge.	The detection limit (sensitivity) varies between kits between 100 – 1000 copies / ml. Factors affecting test result include but is not limited to – quality and type of sample/s collected, duration since onset of disease, appropriate transport in cold chain and time to processing since receipt. A negative result does not exclude infection with SARS-CoV-2.
REFERRAL OF SPECIMENS			

Sr.no.	Specimen / test performed	Indications (major)	Limitations
46	**Lepto PCR**	Suspected leptospirosis, 1st week, antibody negative	A negative test does not rule out disease. A positive test to be correlated clinically and with other microbiological tests. Best results when specimens tested the same day of collection. Follow triple packaging while transporting. Transport in cold chain.
47	**Dengue PCR**	Suspected Dengue, 1st week, NS1 Ag and IgM Ab negative	Same as above. Does not speciate.
48	**Throat / nasal swab for H1N1 influenza**	Category 'C' - Patients with Influenza like illness requiring admission / admitted	Positivity is very high early in the course of disease (upto 5 days). Not recommended as a test for monitoring disease. Processing the specimen within 24 hours of collection improves yield

7. SPECIMEN COLLECTION

a. General instructions and Pre-collection activities

(i) Confirm the identity of the patient

(ii) Explain the procedure to the patient and obtain consent (as appropriate)

(iii) For HIV antibody test, provide pre-test counselling and obtain written informed consent in the requisition form for HIV testing (Appendix 2 and 3)

(iv) Wear appropriate PPE.

(v) Prepare patient as required for the collection

(vi) Collect specimens from the actual site of infection where possible

(vii) Collect the specimen aseptically

(viii) Collect at the appropriate time (where recommended) and in adequate quantity (Appendix 1, pg 70)

(ix) Collect in clean, sterile , screw capped containers

(x) Collect prior to the administration of antibiotics for bacterial culture.

(xi) Label the specimen container with date, name, registration number, ward, unit, specimen, and test required.

(xii) Fill the requisition form completely, legibly and sign before transporting to the laboratory. The minimum details required in the requisition form would include:

name, age, gender, registration number, ward, unit, specimen, date of collection, time of collection where applicable, site from where specimen was collected (where applicable), presumptive diagnosis, nature of investigation required. Any other relevant clinical information if provided will be of assistance such as community / hospital acquired and antibiotic administered current / past. Complete residential address in cases of suspected cholera, typhoid, leptospirosis , dengue and suspected ILI should be provided. **Complete residential address,**

telephone number and vaccination details should be provided by COVID-19 RT-PCR/RAT.

(xiii) After collection, close the container and keep in upright position

(xiv) If the outside of the container is contaminated while collection, decontaminate with 70% alcohol or 0.5% sodium hypochlorite (1:10 dilution) wipe.

(xv) Remove PPE and discard in the red bag. Masks are discarded in yellow bag.

(xvi) Wash hands and dry with a clean towel or use an alcoholic hand rub.

(xvii) If during collection / handling / transport the specimen container breaks, evacuate area adjacent, inform sister in charge / place a large absorbent immediately, and instruct labour staff to immediately follow spill control.

(xviii) Specimens which do not satisfy acceptance criteria will be rejected (pages 64, 65).

b. Note

- The type of specimen required, their quantity for the various investigations carried out in the different divisions and their turnaround time are mentioned at the end of this manual.(Appendix 1, pg 70)
- No emergency testing is done at the Virology and Immunology Division and reports are issued as per the turnaround time mentioned in the appendix.
- NO ADDITIONAL INVESTIGATIONS will be performed from the specimen received for a particular investigation.
- Specimens will not be stored for any other investigation.

- No verbal requests will be entertained for testing.
- While collecting invasive specimens including blood, the phlebotomist / staff collecting the specimen should be identifiable on the requisition form.

- In case the specimen has to be added to a medium such as blood culture, bring the blood culture bottle to room temperature before beginning the collection.

8.DISPOSAL OF WASTE GENERATED (in clinical areas only)

- Segregate waste into appropriate colour coded bags / containers
- Discard all blood soaked non plastic items in yellow bags, all used plastics in red bag, and all sharps in sharp waste disposal container.
- Do not disassemble needle and syringe assembly. Discard the assembly in sharp waste disposal can.
- Fill the bags / containers only to $3/4^{th}$ of its capacity.
- Untreated waste should not be stored beyond 48 hrs
- The red and yellow bags and the sharp cans should be tied, labeled, entered in log book and sent to temporary biomedical waste storage room near gate number 7.
- **Guidelines for handling, treatment and disposal of COVID-19 waste at Healthcare Facilities is given below** -

  o Healthcare Facilities having isolation wards for COVID-19 patients need to follow these steps to ensure safe handling and disposal of biomedical waste generated during diagnosis and treatment;

- Keep separate color coded bins (with foot operated lids)/bags/containers in wards (red and yellow) and maintain proper segregation of biomedical waste .

- As a precaution, double layered bags (using 2 bags) should be used for collection of waste from COVID-19 isolation wards so as to ensure adequate strength and no-leaks.

- Collect and store biomedical waste separately prior to handing over to the same CBWTF. Use a dedicated collection bin labelled as "COVID-19" to store COVID-19 waste and keep it separately in a temporary storage room prior to handing over to authorized staff of CBWTF.

- In addition to mandatory labelling, bags/containers used for collecting biomedical waste from COVID-19 wards, should be labelled as "COVID-19 Waste". This marking would enable CBWTFs to identify the waste easily for priority treatment and disposal immediately upon the receipt.

- General solid waste like wrappers of medicine/syringes etc., fruit peel offs, empty juice bottles or tetra packs, empty water bottles, discarded papers, carton boxes of medicines, empty bottles for of disinfectants and any other items which were not contaminated by the patients' secretions, body fluids should be collected separately as per SWM Rules, 2016.

- Maintain separate records of waste generated from COVID-19 isolation wards.

- Use dedicated trolleys and collection bins in COVID-19 isolation wards. A label "COVID-19 Waste" to be pasted on these items also.

- The (inner and outer) surface of containers/bins/trolleys used for storage of COVID-19 waste should be disinfected with 1% sodium hypochlorite solution daily.
- Feces from COVID-19 confirmed patient, who is unable to use toilets and excreta is collected in diaper, must be treated as biomedical waste and should be placed in yellow bag/container.
- However, if a bedpan is used, then faeces to be washed into toilet and cleaned with a neutral detergent and water, disinfected with a 0.5% chlorine solution, then rinsed with clean water.
- Collect used PPEs such as goggles, face-shield, splash proof apron, Plastic Coverall, Hazmat suit, nitrile gloves into red bag;
- Collect used mask (including Triple layer mask, N95 mask etc.), head cover/cap, shoe-cover, disposable linen Gown, non-plastic or semi-plastic coverall in yellow bags.

9. SPECIAL SITUATIONS – HIV ANTIBODY DETECTION AND CD4 COUNT ENUMERATION

- Patients / Direct walk-in clients whose HIV status needs to be determined, go through the process of pre-test counseling, informed written consent, blood collection, testing and post-test counseling.
- HIV counselling is provided for direct walk-in-clients and OPD patients. Once informed consent is obtained, blood samples are collected for HIV testing.

- For indoor patients, an appropriately collected sample should be sent with a properly filled requisition cum consent form for HIV testing (Appendix 2, 3; pg 65, 66)
- For CD4 count enumeration, only patients referred by the ART centre are tested. Clinicians should refer HIV positive patients under their care first to ART centre who after registration at ART will be referred to Virology and Immunology Division for blood collection and testing.
- NO SAMPLE WILL BE ACCEPTED WITHOUT A **COMPLETELY FILLED REQUISITION FORM** (Appendix 2, 3 and 6) . The requisition cum consent form for HIV testing should mention the name, registration number, age/gender, ward/ OPD number, date and time of collection, name of the unit the patient belongs to, occupation of the patient, nature of specimen, and relevant clinical indication for testing and should be duly signed by the clinician. For HBsAg / anti-HCV testing the requisition form should mention the name, registration number, age/gender, date and time of blood collection, ward/ OPD number, name of the unit the patient belongs to, clinical indication for testing, nature of specimen and investigation required.

Consent for HIV testing

- Ensure that an informed written consent is taken after pre-test counselling for HIV testing.

- The consent form is available in English and Marathi (Appendix 2 and 3). Choose the language that the patient understands or have it understood if both are not applicable.
- Pre and post-test counselling is mandatory for all patients undergoing HIV testing. For indoor patients, it can be carried out by trained resident doctors, staff nurses, medical social workers, etc. Only if the patient is willing for HIV testing, his/her blood should be collected.
- In case of minors, the consent should be obtained from the parents/guardians.
- In case of unconscious patients, where there is a need for diagnosis of HIV for management of the patient, consent should be obtained from the parents/ spouse/ closest relative available at that time.
- In case no attendant is available, the test if necessary for management may be carried out on recommendation of two attending doctors.

10. SPECIMEN COLLECTION - BLOOD – [FOR SEROLOGY, VIROLOGY AND IMMUNOLOGY AND MOLECULAR DIAGNOSTICS]

- Blood collection is performed only by well-trained experienced phlebotomists (Laboratory technicians / Doctors).
- Ensure that the patient is at least 2 hours fasting before specimen collection.

- Requirements – Gather material required for collection and biomedical waste disposal. This includes -

Tourniquet, Alcohol wipes, Sterile syringe and needle (21 G preferably) or appropriate evacuated tube sets , cotton ball, gloves, alcoholic hand rub solution, collection container - preferably pre-labelled [clean / sterile , dry test tube or evacuated tubes - red cap for plain blood and purple cap for EDTA], sharps can, requisition form, red bag and yellow bag.

- If multiple collections are done using the same gloves, and if the gloves are visibly clean, the same pair of gloves can be used provided the gloves are disinfected after every collection using 70% alcohol/ alcoholic hand rub.
- In case there is contamination with blood, the gloves should be removed immediately and discarded in the red bag and replaced with new pair of plastic and latex gloves.

Procedure

- Help the patient sit comfortably on a chair with an armrest / or lie down on a bed/couch.
- Use alcoholic hand rub to disinfect your hands.
- Wear plastic and clean latex gloves. Also wear a plastic apron if required.
- Place absorbent material (cotton/gauze piece) below the patient's elbow to avoid soiling due to any leakage.
- Inform patient about the collection and the discomfort that is likely to be felt [a small prick like an insect bite].

- Pre label the collection device with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection.
- Tie a tourniquet above the site of blood collection to make the vein prominent. [This is usually above the patient's anterior cubital fossa on the forearm].
- Instruct the patient to clench his/her fist while collection is on.
- Disinfect the site of collection [patient's] with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol].
- After use, discard the alcohol swab in the yellow bag.
- Take a new sterile needle [preferably 21 G for an adult and 22 G for a child] and syringe / sterile evacuated tube set in front of the patient. The needle is attached to the syringe.
- Discard the paper/plastic cover of the syringe and needle in the blue bag.
- Insert the needle aseptically into the vein at an angle of 45 degrees.
- Allow blood to flow and collect 3-6 ml/ as per evacuated tube capacity.
- Release the tourniquet.
- Tell the patient to release the clenched fist.
- Withdraw the needle slowly and place a dry cotton swab at the puncture site.
- Ask patient to keep the elbow flexed until blood flow stops. [Usually 2-5 minutes]
- If syringe has been used, transfer the blood gently along the wall without squirting into appropriate pre-labelled collection container.

- Discard in the designated sharp can.
- Where collection is done at the laboratory, ask patient to leave after checking that there is no bleeding from the puncture site and to discard the used cotton swab in the yellow bag.
- Any used cotton / gauze should be discarded in yellow bag.

11. BLOOD – FOR CULTURE [AEROBIC / FUNGAL]

- Both conventional and BACTEC blood culture bottles should be stored in the refrigerator compartment (2 -8 ° C) before use.
- Bring to room temperature prior to adding blood.
- In case of delay in transport to laboratory, store at room temperature.
- Blood collection is performed only by well-trained experienced phlebotomists (Laboratory technicians / Doctors).
- Collect blood during fever / spike phase
- **Collect 7-10 ml in adults, 3-5 ml in children and 1-2 ml in neonates ensuring the required volume in each set (if available)**.
- **Each time, collect blood in two blood culture bottles** (a set) with each bottle having a volume of blood as mentioned in the point above.
- **Number of specimens** - Collect twice from two different sites within an hour of each other or two specimens over 24 hrs ensuring the volume as mentioned above at each collection
- Requirements – Gather material required for collection and biomedical waste disposal. This includes -

Tourniquet, Alcohol wipes, Betadine / Chlorhexidine solution, Sterile syringe and needle (21 G preferably) or appropriate evacuated tube sets , cotton ball, gloves, alcoholic hand rub solution, **prelabeled container - blood culture bottle with appropriate medium** [large (100 ml) for adults and small McCartney bottles for children / BACTEC aerobic plus and BACTEC Peds plus] brought to room temperature if refrigerated and with the top disinfected with alcohol wipes , sharps can, requisition form, red bag and yellow bag.

Procedure

- Follow instructions as mentioned under collection of blood with the following modifications.
- Labeling - Pre label the blood culture bottle with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection.
- Site disinfection - Disinfect the site of collection with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol or chlorhexidine]. After use, discard the alcohol swab in the yellow bag.
- Follow this with disinfection with alcoholic chlorhexidine (preferred) / povidone iodine in a circular motion beginning from centre and moving out. Allow to dry. Discard the cotton swab in yellow bag.

- Take a new sterile needle [preferably 21 G for an adult and 22 G for a child] and syringe / evacuated tube with holder. The needle is attached to the syringe / evacuated tube.
- **Collect adequate volume**
- Transfer the blood gently and aseptically into the blood culture bottle along the wall without squirting. Mix the contents well by placing on a horizontal surface.
- Send the specimen immediately to laboratory.

12. BODY FLUIDS FOR CULTURE

(Ascitic / peritoneal fluid, pleural fluid, pericardial fluid, synovial fluid etc.)

Responsibility: Clinician

- Disinfect the site of collection using alcoholic chlorhexidine / povidone iodine
- Wait for it to dry
- Inform the patient of the procedure
- Using aspetic precautions, collect in a sterile, screw capped container available for the same which is labeled appropriately
- Collect 2-5 ml where possible
- Transport immediately to laboratory
- In case of delay in transport, store at room temperature only. Do not refrigerate.

13. CSF FOR CULTURE

Responsibility: Clinician

General instructions:

- The collection of CSF is an invasive technique and should be performed by experienced clinicians under aseptic conditions
- It is unsafe to do lumbar puncture in case of increased intracranial pressure
- LP should not be performed through infected skin as organisms can be introduced into the subarachnoid space (SAS)
- Clinician should explain the procedure to patient / relative if patient comatose in detail
- **The container should be sterile, screw capped** (available from general stores) labeled appropriately [see general instructions]. **DO NOT COLLECT IN PENICILLIN BULBS SINCE THEIR STERILITY IS NOT MAINTAINED. DO NOT COLLECT IN GEL TUBES (Yellow cap)**
- Labeling – as in 'blood'
- Usually, 3 tubes of CSF are collected for biochemistry, microbiology, and cytology.
- If only one tube of fluid is available, it should be given to the microbiology laboratory.
- If more than one tube (1 ml each) is available, the second or third tube should go to the microbiology laboratory.
- Avoid exposure of CSF to excessive cold, heat or sunlight.

- IN CASE OF DELAY IN TRANSPORT TO LAB AFTER COLLECTION, STORE AT ROOM TEMPERATURE OR INCUBATOR ONLY. **DO NOT REFRIGERATE**.

Requirements: The kit for collection of CSF should contain:

- skin disinfectant
- sterile gauze and Band-Aid
- lumbar puncture needles: 22 gauge/3.5"for adults;
- 23 gauge/2.5" for children
- sterile screw-cap tubes
- Sterile screw capped tubes
- sterile gloves

Procedure

- **Analgesia – as recommended**
- **Positioning**
  - Position the patient at the edge of a firm bed and on one side rolled up into a ball.
  - The neck is gently ante-flexed and the thighs pulled up toward the abdomen; the shoulders and pelvis should be vertically aligned without forward or backward tilt
  - LP is performed at or below the L3-L4 interspace.
  - An alternative to the lateral recumbent position is the seated position. The patient sits at the side of the bed, with feet supported on a chair. The patient is instructed to curl forward, trying to touch the nose to the umbilicus.

- A disadvantage of the seated position is that measurement of opening pressure may not be accurate.

Procedure

- Perform hand hygiene and wear sterile latex gloves
- Disinfect the skin with povidone-iodine or similar disinfectant and drape the area with a sterile cloth
- Inject local anaesthetic as recommended.
- Wait for 5-15 minutes
- The LP needle (typically 20- to 22-gauge) is inserted in the midline, midway between two spinous processes, and slowly advanced. The bevel of the needle should be maintained in a horizontal position, parallel to the direction of the dural fibres and with the flat portion of the bevel pointed upward; this minimizes injury to the fibres as the dura is penetrated.
- When lumbar puncture is performed in patients who are sitting, the bevel should be maintained in the vertical position.
- In most adults, the needle is advanced 4–5 cm (11/2–2 in.) before the SAS is reached; the examiner usually recognizes entry as a sudden release of resistance, a "pop."
- If no fluid appears despite apparently correct needle placement, then the needle may be rotated 90°–180°.
- If there is still no fluid, the stylet is reinserted and the needle is advanced slightly.
- Once the SAS is reached, a manometer is attached to the needle and the opening pressure measured.

- CSF is allowed to drip into collection tubes; it should not be withdrawn with a syringe.
- **Volume - 2-4 ml** of CSF should be collected, the rate of collection should be slow, about 4-5 drops a second [1 ml minimum volume required for culture]
- Prior to removing the LP needle, the stylet is reinserted to avoid the possibility of entrapment of a nerve root in the dura as the needle is being withdrawn; entrapment could result in a dural CSF leak, causing headache.
- Following LP, the patient is customarily positioned in a comfortable, recumbent position for 1 h before rising,
- When the procedure is completed, the needle is removed and an adhesive bandage is placed over the injection site.
- Label the specimen as described earlier.
- Transport to the laboratory as soon as possible.

14. EAR SWAB

- Use sterile swab stick
- Collect under direct vision
- Do not instill antibiotic / antiseptic into the ear prior to collection
- Allow the swab to soak in the exudate for 10 seconds
- **Place in prelabeled sterile container (plugged / screw capped test tube)** and transport immediately.

15. EYE SWAB (CORNEAL/ CONJUNCTIVAL)

- Moisten the swab in sterile normal saline
- Hold the swab parallel to the cornea and gently rub the lower conjunctiva
- **Place in prelabeled sterile container (plugged / screw capped test tube)** and transport immediately.

16. COLLECTION OF LOWER RESPIRATORY TRACT SPECIMENS

Types of specimen:

Lower Respiratory Tract Specimens include:

a. Sputum –expectorated
b. Sputum - induced
c. Bronchial washings
d. Bronchial aspirate
e. Bronchial brushing
f. Broncho alveolar lavage [BAL]
g. Mini-BAL
h. Endotracheal aspirates
i. Tracheal swabs
j. Protected catheter brush specimen
k. Transthoracic aspirates
l. Trans tracheal aspirate
m. Open Lung biopsies

Responsibility: Clinician (or nursing assistant depending on invasiveness of procedure)

a. Sputum –expectorated

Requirement:

- Patients without complaints of cough with expectoration should preferably not be referred for sputum examination.
- **For culture - The container** should be sterile, wide-mouthed, screw-capped with a capacity of approximately 15-20 ml and labeled. The container can be procured from 7thfloor, Clinical Bacteriology Div / general stores. The procedure of collection should be explained to the patient. This includes:

Explaining the difference between saliva (spit) and sputum.

Explaining the cough etiquette and its importance

For sputum microscopy(acid fast bacilli)- clean, screw capped container provided by DOTS centre (5th floor, CVTS bldg.)

- Collection:

Volume – 2-5 ml

Number of specimens: One for bacterial culture

Two (one early morning and one spot) for sputum AFB/Xpert assay examination

Collection should be done in a well-ventilated area away from people, especially children.

The patient should first rinse his/her mouth with plain water.

The patient should open the container without contamination, breathe slowly and deeply, bend forward and generate a deep cough.

Collect the expectorant in the container by pressing the rim of the container under the lower lip to catch the entire expectorated cough sample.

After collection, the cap of the container should be tightly screwed.

Any spilled material on the outside should be wiped off with a tissue moistened with 0.5 % sodium hypochlorite (1:10 dilution, prepared daily) or alcohol, and care should be taken not to let any disinfectant enter the container.

If the collection is done at home, visible contamination should be wiped off with house hold bleach.

- It should be ensured that the sputum sample is of good quality. A good quality sputum sample is thick, purulent and sufficient in amount (2-3ml).
- Fill the form and send sample immediately to lab.

Sputum – Induced

- When sputum production is scanty, induction with physiotherapy, postural drainage, or nebulized saline may be effective.
- This procedure should be carried out in an area which is isolated and preferably under negative pressure or well ventilated without other humans around.
- Allow the patient to breathe aerosolized droplets of a solution containing 15% sodium chloride and 10% glycerin for 10 minutes or until a strong cough reflex is generated.
- Collect the sputum thus generated (which tends to be watery) **in a sterile screw capped labeled container (as for sputum above)** and send to the laboratory immediately along with the duly filled requisition form.

- Mention that the specimen is induced sputum in order to avoid specimen rejection.

b. **Bronchial washings**

- Bronchial washings are collected in a similar fashion to bronchial aspirate (see below), but the procedure involves the aspiration of small amounts of instilled saline from the large airways of the respiratory tract.
- **Container – Sterile screw capped test tube**

c. Broncho alveolar lavage (BAL) culture

- The sampling area is selected based on the correspondent area of the infiltrate on chest radiograph or by the visualization of a sub segment containing purulent secretions.
- A volume of sterile saline is instilled and then gently aspirated. (approximately 100 ml)
- Approximately 5 ml lavage is to be sent to the laboratory for microbiological examination.
- **Container – Sterile screw capped test tube**

d. Endotracheal aspirate

- Indication - in intubated patients with suspicion of pulmonary infection
- Position the tip of the bronchoscope close to the segmental area corresponding to radiographic infiltrates.
- Instill 3 aliquots of 50 mL or 5 aliquots of 30 mL saline
- After the injection of each aliquot, gently aspirate through the suction channel.

- Send atleast 10 ml of the aspirate for microscopy and culture.
- **Container – Sterile screw capped test tube**

e. Bronchial aspirate

These are collected by direct aspiration of material from the large airways of the respiratory tract by means of a flexible bronchoscope. Approximately 5 ml lavage is to be sent to the laboratory for microbiological examination.

Specimen container for Xpert MTB/RIF assay is the 50 ml, conical, graduated, sterile, screw capped, Falcon tube provided by DOTS center, 5th floor, CVTS building.

17. COLLECTION OF UPPER RESPIRATORY TRACT SPECIMENS

Types of specimen:

- throat swab
- nasopharyngeal swab

Requirement:

- Sterile swab
- **Container** - Sterile test tube , screw capped / cotton plugged to place the swab
- Clean tongue depressor
- Source of light

General instructions

- Follow standard precautions

- In suspected cases of diphtheria and flu, swabs should be collected both from the throat and the nose
- In case of flu, use the special swab provided with the viral transport medium (VTM). Maintain cold chain in triple pack while transport.
- Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction

Procedure:

- Perform hand hygiene.
- Wear appropriate mask / respirator for personal protection.
- Use a face shield.
- Wear clean / sterile gloves.
- Ask patient to open his / her mouth without putting out his tongue and to say 'Ahhhhh….'
- While the patient is saying 'Ahhhhh', press down the outer two third of tongue with tongue depressor, using the left hand, enabling the tonsils and back of the throat to become visible.
- Introduce the swab with right hand between the tonsillar pillars and behind the uvula, while avoiding touching the tongue, cheeks, uvula, or lips.
- Rub the swab firmly against the inflamed part for 5 seconds while turning it round
- In case of suspected diphtheria, swab the membrane if present and If nothing abnormal is seen, swab the tonsils, the fauces and the back of the soft palate

- Take two swabs and immediately plug the same in sterile test tubes
- Specimens should be transported to the laboratory immediately after labelling and properly filling up the requisition form.

Nasopharyngeal Swab Collection Procedure

Ask the patient to tilt the head slightly (70 °).

Label the collection tube (VTM) with patient's name and registration number and date of collection.

Insert the swab gently into either nostril, passing it into the posterior nasopharynx (see Figure 1).

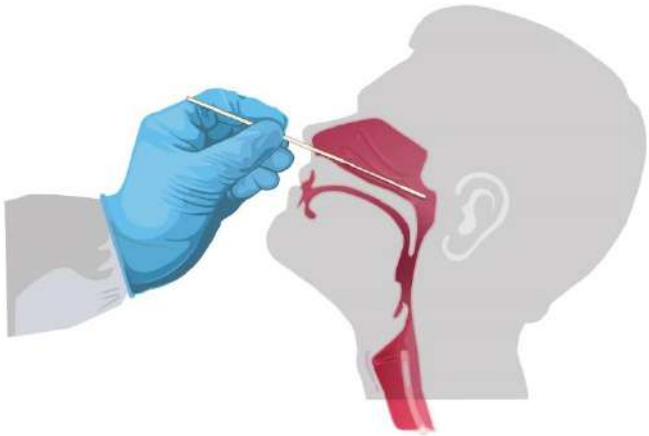
Insert the swab into the nostril, parallel to the palate until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx.

The swab should reach a depth equal to the distance from the nostrils to the outer opening of the ear.

Rotate swab by firmly brushing against the nasopharynx several times.

Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline.

Break swab at the indicated break line and cap the specimen collection tube tightly



https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Oropharyngeal Swab Collection Procedure

- Swab the posterior pharynx, tonsils, and other inflamed areas. Avoid touching the tongue, cheeks, and teeth with the swab when collecting specimens.
- Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline.
- Break swab at the indicated break line and cap the specimen collection tube tightly.

Nasal Swab Collection Procedure

- Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril

18. Ophthalmic specimens - corneal scrape and conjunctival scraping

To be collected only by ophthalmologist.

After anaesthetizing the eye with local anaesthetics, retract the lid with retractor.

Using the blunt edge of sterile scalpel blade, scrape the ulcerated area away from the pupillary area.

Wipe the scrapings on a sterile swab stick wetted with broth

Collect more scrapings in similar way for smear and KOH mount.

19. PUS

- Aspirate pus through a sterile syringe and needle where possible.
- **Transfer a portion (1-2ml) to a screw capped sterile container(test tube)**
- **For anaerobic organisms**, transfer specimen to Robertson's cooked meat medium for culture. The medium is available from media room, Department of Microbiology, 7th floor, MSB.

20. SKIN, NAIL AND HAIR – FUNGUS

(Collect skin scraping, hair and nail clippings in a petridish / test tube and maintain at room temperature)

a) Skin scrapings

- Identify the site of lesion from where collection is to be made.

[An appropriate lesion is peripheral, erythematous, growing margins of typical ring worm lesion.]

- Inform the patient about the procedure.
- Collect specimen with strict aseptic precautions.
- Make patient sit comfortably.
- Clean the identified lesion thoroughly with 70% alcohol to remove the surface bacterial contamination.
- Using sterile scalpel blade surface collect multiple scrapings from the identified lesion preferably from the edge of lesion including the adjacent healthy skin.
- **Collect the specimen in petri dish, filter paper or clean paper.**

b) Nail

- Clean the affected nail with spirit
- Collect debris under the nail with scalpel in petridish
- Pick up flakes after wetting loop with sterile saline from petridish for processing
- If nail is avulsed then it should be cut in small pieces for processing.

c) Hair

- Hair should be collected from areas of scaling or alopecia
- Clean the affected area with spirit
- With sterilized forceps, pluck hair or stubs (at least 10-12) in grey patch or scrape with scalpel in black dot type of hair infection.

d) Skin Biopsy

- Decontaminate skin with 70% methylated spirit
- Select the edge of the lesion
- Take a biopsy with autoclaved instrument under all aseptic measures
- Cut biopsy tissue in small pieces and crush in mortal and pestle.

e) Mycetoma granules

- From suspected mycetoma, look for granules in the lesions using hand lens.
- Wash the granules in several changes of sterile distilled water
- Crush the granules and then inoculate.
- If granules are absent collect the purulent/necrotic material**.**

21. STOOL

- Collect fresh stool specimen in a decontaminated and well rinsed bed pan. **Transfer one teaspoonful** to the appropriate screw capped container.

22. URINE – CLEAN CATCH

Provide adequate instructions on what to collect (mid-stream) and how much to collect **(5ml**) and container (screw capped sterile container) to be used, to patients for **clean catch** mid-stream urine specimens. In case there is likely to be a delay in transport, refrigerate the specimen (4°C)

Men: Retract the prepuce and clean the urethral meatus with soap and water. Collect mid-stream urine.

Women: Clean the periurethral area with soap and water, movement

being directed front to back. Repeat twice. Collect mid-stream urine.

Urine –catheterized

- Decontaminate / Disinfect catheter specimen port with alcohol wipe.
- Using a sterile syringe and needle collected 5 ml urine form catheter specimen port.
- Transfer the specimen to the appropriate urine container (screw capped test tube, sterile)
- In case there is likely to be a delay in transport, refrigerate the specimen (4°C)

Urine – Suspected tuberculosis

- Early morning urine , **25-30 ml**, on three consecutive days

23. WOUND SWAB

- Not a good quality specimen
- Aspirated fluid / tissue preferred
- If swabs need to be collected, use a sterile swab.
- Collect two swabs.
- Cleanse the wound with sterile distilled water / normal saline wipes.
- Place the swab in the wound / purulent area, rotate gently for 10 seconds allowing the secretions to be soaked.
- Place in a sterile labeled container (test tube, plugged / screw capped) aseptically and transport immediately to lab.

24. NEEDLE STICK INJURY PROTOCOL

Needle stick injury, while collecting/transporting/handling/disposing specimens / collection devices, is an indication for post exposure prophylaxis (PEP).

Procedure to be followed when exposure has occurred

- Wash the area with soap and water
- Avoid squeezing or milking the wound
- Do not use caustic agents, such as bleach
- Inform your superior and consult ART (anti retroviral therapy) center , Ground floor, MSB, during routine hours for PEP drugs.
- After routine hours, consult MICU (2^{nd} floor, main hospital building) for PEP drugs
- The medical officer at each of these places will determine risk i.e. Type of exposure and Infection Status of Source and decide on treatment
- Get Lab tests done and follow up in 3-6 months
- Follow medical officer's advice for duration of PEP.
- It is important to initiate PEP as early as possible and within 72 hours.

25. SPILL PROTOCOL

For spills with blood and body fluids

- Clear the area of spill and start spill containment
- Instruct the housekeeping staff on the protocol which is as follows:
- Don appropriate personal protective equipment (impervious gown, gloves, face shield or goggles as appropriate and boots if spill is large.).

- Wear heavy duty gloves and then pick up any broken glass with the help of forceps and discard into a sharps container.
- Cover spill with paper towels / absorbent (gauze) and allow soaking.
- Discard in yellow bag.
- Cover spill again with paper towels / absorbent (gauze).
- Squirt disinfectant (1% Na hypochlorite; 1:5 dilution) onto absorbent with circular motion, from the outside towards the centre.
- Allow to stand for at least 10 minutes.
- Discard used paper towels/ absorbent (gauze) in the yellow biohazard bag.
- Mop the area with 1% Na hypochlorite.
- Disinfect the heavy duty gloves and forceps with 1% Na hypochlorite before storage, wash well in running water and store dry.

26. SPECIMEN TRANSPORT

- The transport of specimens should be done as soon as possible to the respective divisions, preferably within 2 hours of collection along with the completely filled and signed requisition form. Check specimen acceptance timings.
- Place the specimen container in a tray / container in such a manner that it remains upright and does not spill/fall. Do not transport specimens in apron or shirt pockets.
- The person transporting the specimen should be instructed as to the location for the test and provided with gloves by the clinician and sister in charge respectively.

- If specimens are not transported as per requirement, they may be rejected. (see rejection criteria below)
- The requisition forms should accompany the specimen and should not be placed in the same tray as the specimen. Do not wrap the requisition form around the specimen container.
- The specimens and forms should be transported in a separate container / tray.
- REQUISITION FORMS SOILED WITH SPECIMEN WILL NOT BE ACCEPTED.
- Specimens for molecular diagnosis except CBNAAT for TB should be transported only in cold chain.

27. STORAGE OF SPECIMENS (TEMPORARY)

- In case of an anticipated delay in the transport of blood specimens beyond 4 hours, allow the blood to clot [for investigations requiring serum] and then store in the refrigerator and send the next day. The same should then be clearly mentioned on the requisition form.
- Other specimens that can be stored in the refrigerator but not beyond 24 hrs. include– Urine for culture, Sputum for AFB , skin / hair / nails for mycology
- **Specimens that cannot be stored in the refrigerator** – blood and all body fluids for culture.

In case of a delay in transporting these specimens, keep them at room temperature.

- Specimens that need to be transported immediately to the laboratory – blood for culture, specimens collected on swabs, stool specimen for parasites and cholera, specimens for detection of anaerobes and CSF from suspected cases of meningitis, specimen for COVID-19 RAT/RT-PCR.

28. SPECIMEN RECEIPT AND ACCEPTANCE

- The specimens are accepted at the reception counter for each division except for COVID-19 RT-PCR and RAT. Refer to specimen acceptance timings above.
- This section is manned by a trained laboratory technician and assistant / laboratory attendant who also guides the patients for other investigations if required.

- The designated person checks transport conditions and instructs for corrections if deviations found.
- Validate the details on the requisition form with the specimen and the label on the container.
- If appropriate, the dispatch is signed
- Acceptance is based on the following criteria being satisfied:

Specimen acceptance criteria

- Appropriate specimen
- Appropriately labelled container
- Appropriate volume
- Appropriate transport (including PPE provision)
- Completely filled and signed requisition form
- No breakage / leakage / soiling of container / requisition form
- Details on label of specimen container, the specimen and requisition form match

29. CRITERIA FOR SPECIMEN REJECTION

- Incomplete requisition
- Soiled/ blood stained requisition form (specimen is accepted; new form is asked)
- Written consent not taken for HIV testing
- Mismatch between details on requisition form and specimen container
- No signature of clinician on requisition form
- Specimen transport time has exceeded two hours for urine culture

- Leaking or broken specimen container
- For culture, open containers
- For culture, specimen in formalin
- Specimen in wrong container
- Blood sent for culture in any other container other than blood culture bottle.
- Any sample sent for culture in penicillin bulb / yellow capped evacuated tube
- Insufficient specimen quantity (except invasive specimens)
- Hemolysed blood specimen for serology
- Lipaemic blood specimen for serology
- For culture, cotton plug contaminated with specimen
- For culture, Foley's tip.
- Dried swabs sent for culture
- Saliva instead of sputum for culture

30. **REPORT DISPATCH**

The reports are delivered through various modes:

- HIV reports are given to the respective direct walk-in clients/OPD patients after post-test counselling by the counsellor.
- HIV reports of ante natal clinic (ANC) patients are handed over to the counsellor working under the PPTCT (Prevention of parent to child transmission) program.
- HIV reports of indoor patients - HIV positive reports are directly handed over to the patient by ICTC counsellor after post-test

counselling in the ward. All HIV negative indoor patient reports are dispatched to the referring unit.

- CD4 and HIV viral load reports are handed over to the Anti-Retroviral Therapy Centre counsellor.
- HBV and HCV viral load reports are handed over to Gastroenterology department.
- For outdoor patients whose specimens have been processed in any division [other than for detecting HIV antibodies or HIV viral load], reports are handed over directly to the patient / representative on producing the relevant copy of the request.
- For indoor patients whose specimens have been processed for any test other than those mentioned previously, reports are dispatched to the respective wards by an identified dispatch peon.
- Nikshay entry of all Xpert/MTB Rif assay and microscopy reports is done daily by laboratory technician.
- Appropriate log of report dispatch and delivery is maintained.
- Duplicate reports are issued on request of the referring clinician/ patient. The report is clearly marked as duplicate.
- COVID-19 RT-PCR reports of OPD patients will be dispatched to Gymkhana , the next day.
- COVID-19 RTPCR and RAT details are uploaded on ICMR portal @cvstatus.icmr.gov.in which is password protected.

31. COMPLAINTS

For any complaints pertaining to any of the services offered, a note maybe sent anytime to the HOD to facilitate correction as required and

improvement of services. Clinicians are also requested to fill the annual feedback forms with relevant suggestions for improvement.

32. REFERENCES

- Procop GW, Church DL, Hall GS, Janda WM, Koneman EW, Schreckenberger PC, Woods, GL. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 7th edition. Philadelphia: Wolters Kluwer Health; 2017
- Tille P. Bailey & Scott's Diagnostic Microbiology. 14th edition. St. Louis: Elsevier Mosby; 2014
- World Health Organization Chapter 2 Collection and Transportation of Clinical Specimens In Blood Safety and Clinical Technology / Guidelines on Standard Operating Procedures for Microbiology available online @ http://www.searo.who.int/EN/Section10/Section17/Section53/Section482_1779.htm
- GOI, MoHFW, No 7-165/2016/NVBDCP/DEN – Dated 9th June 2016
- Icmr.gov.in for all COVID-19 related documents.

APPENDIX 1
Tests offered and their TAT (Next page)

Division	Test Offered	Volume	TAT After specimen receipt
Virology and Immunology Division	HIV testing for indoor patients and antenatal mothers	3-6 ml blood in a plain test tube or Red / yellow cap evacuated tube along with requisition form	Next working day after 2 pm
	HIV Counselling and testing for direct walk in clients and OPD patients	Patient referred to ICTC	Same day after 3 pm (for specimens collected before 12 pm) Next working day after 2 pm (for specimens collected after 12 pm)
	HBsAg testing	3-6 ml blood in a plain test tube or Red / yellow cap evacuated tube along with requisition form	Next working day after 2 pm
	HCV antibodies	3-6 ml blood in a plain test tube or Red / yellow cap evacuated tube along with requisition form	Next working day after 2 pm
	CD4 count estimation	3-6 ml blood in a EDTA evacuated tube along with requisition form	Next working day 12 pm

	HIV viral loads for patients referred from ART	Patients referred to ICTC for collection along with requisition form. 3- 6 ml blood in EDTA evacuated tube	14 working days
	Viral load - HBV/ HCV (as per availability)	Patients referred to ICTC for collection along with requisition form. 3- 6 ml blood in EDTA evacuated tube	HBV and HCV – Tests are referred to LTMGH NVHP laboratory.
Clinical Bacteriology The container for collection should be clean, sterile and screw capped or plugged and appropriately labelled.	Microscopy – Gram's stain, Albert's stain	**1.0 ml** **Critical specimens** – **CSF**, Tissue / swab for **gas gangrene**, Tissue / swab for **Diphtheria,Pancreatic fluid, Brain abscess, Ocular** specimens	1 hr
	Microscopy – Gram's stain	Specimens other than above	4 hrs
	Hanging Drop	1 ml	30 minutes
	Aerobic culture	At least 1 ml except blood culture [refer section]	24 – 96 hrs
	Antibiotic Sensitivity Test – aerobic bacteria	NA	72 hrs – 5 days
	Anaerobic culture	Sterile Swabs – soaked in exudates Tissue – NA Pus – at least 1 ml	72 hrs. – 5 days
	Surveillance cultures	Exposure plates for clean rooms	24 hrs. for aerobic bacteria

		(such as operation theatres) and swabs from environmental and clinical contact surfaces as appropriate	72 hrs. for sporing anaerobes 5 days – 2 weeks to rule out fungal contamination
Molecular Diagnosis	COVID-19 RT-PCR Zika virus PCR	NP swab + OP swab / Nasal + throat swab / Nasal swab / Sputum / BAL Blood	12 – 24 hrs from receipt of specimen 72 hrs
Mycology	Microscopy	Nail hair biopsy	24 hours
	Microscopy	Other	4 routine working hrs
	Culture and identification	At least 3 ml if liquid	48 hrs. – 1 month
	AFST for yeasts (as per availability)	----------	48 hrs after culture positivity
Myco-bacteriology	Microscopy	Any	24 hrs. from acceptance
	Culture - MGIT	At least 3 ml in case of non-tissue specimens	21 days – 42 days
	DST - MGIT	----------	18-26 days after culture positivity
	Xpert MTB/RIF assay	2 ml for any specimen in Falcon tube procured from division	≤ 48 hrs.
Parasitology The container should be clean and screw capped.	Microscopy	1 tsp stool specimen	4 routine working hrs
	Malaria Antigen Detection	Whole blood in EDTA evacuated tube (3 ml)	2 hrs

	Opportunistic protozoon parasites	5 ml / 1 gm of any specimen	4 working hrs
Serology	VDRL/RPR	3-6 ml blood sample in a plain test tube/ Red / yellow capped evacuated tube	4 hrs
	Widal		24 hrs
	Dengue antibody rapid		4 hrs
	Dengue – NS1 antigen (rapid)		4 hrs
	Dengue NS1 antigen (ELISA)		72 hrs
	Dengue IgM (ELISA)		72 hrs
	Rapid – Lepto IgM		4 hrs
	ASO		4 hrs
	RF		4 hrs
	Chik IgM		72 hrs
	SARS-CoV-2 IgG ELISA		7 days
	Dengue and Lepto PCR pH1N1 PCR	6 ml blood collected in EDTA evacuated tube Throat / Nasal swab in VTM	Result from Mol Diagnostic Lab – Kasturba
	COVID-19 RAT	NP swab collected in buffer provided with kit	30 minutes from receipt
Emergency Laboratory	Critical specimens / critically ill patients **Microscopy** Gram's stain Albert's stain Indi Ink for Cryptococcus Stool-Hanging Drop **Culture** – inoculation only	1.0 ml	1 hr for critical specimens 2 hrs for others

	Malaria - RDT	3.0 ml (whole blood/serum)	2 hrs
	Leptospira IgM - Rapid Dengue NS1 Ag – Rapid Dengue IgM,IgG Ab - Rapid	3 – 6 ml blood in plain tube / evacuated tube with red top	2 hrs

APPENDIX 2

Municipal Corporation of Greater Mumbai
Seth G.S. Medical College & K.E.M. Hospital
Department of Microbiology
HIV Antibody Test Requisition Form

Name : Age: Gender: M / F

Reg. No: Ward No: Unit:

Diagnosis: Occupation:

Type of Primary Specimen: Venous Blood

Date of Specimen Collection: Time of Specimen Collection: am/pm

Sign of Clinician:

Consent for HIV Testing

This is to state that I have been counseled about the HIV test and have been explained about the implication of the test results. All the details pertaining to HIV, its transmission, prevention, testing procedure, its limitations and interpretation of results have been explained to me in a manner that I can understand.

I, hereby, give my consent for the test to be conducted on me / my ward in order to ascertain my / my ward's HIV serostatus.

Signature of Client / Parent_____ Date:_____

Counseled by (Name and signature)_____ Date:_____

एच.आय.व्ही. चाचणीसाठी लिखित संमती

मी याद्वारे नमूद करतो/ करते की, माझ्या/ माझ्या पाल्याच्या रक्ताच्या नमुन्यावर एच.आय.व्ही. संबंधाने करावयाच्या चाचणी बाबत माझ्याशी विचार-विनर्श करण्यात आला असून मला त्या संबंधीची माहिती पुरविण्यात आली आहे. एच.आय.व्ही. संसर्गाबाबत करण्यात येणाऱ्या चाचणीच्या संभाव्य निष्कर्षाबाबत मला समजाविण्यात आले आहे. त्याचप्रमाणे, एच.आय.व्ही. म्हणजे काय, त्याचा संसर्ग कसा होतो, त्याचा प्रतिबंध कसा केला जातो, चाचणीची प्रक्रिया, तिची मर्यादा आणि चाचणीच्या निष्कर्षांचा अर्थ आदि संबंधी सर्व माहिती, मला समजेल अशा पध्दतीने स्पष्टपणे सांगण्यात आली आहे.

माझ्या/ माझ्या पाल्याच्या एच.आय.व्ही. संसर्गाची पातळी निश्चित करण्यासाठी माझ्या/ माझ्या पाल्याच्या रक्ताच्या नमुन्यावर चाचणी करण्यासाठी मी याद्वारे माझी संमती देत आहे.

आरोग्याची/ पालकाची स्वाक्षरी_____ दिनांक-_____

समुपदेशकाचे नाव व स्वाक्षरी_____ दिनांक-_____

FOR LABORATORY USE ONLY

Date of Receiving Specimen: Time of Receiving Specimen: am/pm

Lab No: Received By: Sign:

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APPENDIX 3

Municipal Corporation of Greater Mumbai
Seth G.S. Medical College & K.E.M. Hospital
Department of Microbiology
HIV Antibody Test Requisition Form

Note:

1. Consent obtained for carrying out procedures in hospitals does not include consent for HIV testing. Separate consent has to be taken for a HIV test.
2. Informed consent of parents / guardians is required prior to testing of minors for HIV.
3. Informed consent can be given by persons suffering from mental illness depending upon their current condition as assessed by the designated authority; else, consent of their guardian should be obtained prior to HIV testing. (Referral to trained mental health professionals should be made if required).
4. In case of unconscious patients, where an HIV test is in the best interest of the patient for HIV management, consent should be taken from one of the following: parents, spouse or closest relative or in case of non-availability, the HIV test may be carried out on recommendation of two attending medical practitioners.
5. Non-voluntary disclosure of confidential medical information including HIV status may be made in cases where such disclosure is medically beneficial for the client or in case where there is a significant risk of HIV transmission to an identifiable partner. The disclosure cane me made to a health care worker who is directly involved in the care or treatment of the client. The disclosure can also be made if there is a threat to the life of the client (suicidal ideation) or his / her partner or spouse (partner notification)

टीप:

1. रुग्णालयात विविध चाचणी/तपासणी करण्यासाठी घेतल्या जाणा-या सर्वसामान्य संमती मध्येच एच.आय.व्ही संबंधीच्या संमतीचा समावेश नसतो. एच.आय.व्ही चाचणीसाठी त्यासंबंधीची वेगळी संमती घेण्यात यावी.
2. अज्ञान व्यक्तींच्या संदर्भातील चाचणीसंबंधीची आवश्यक संमती, अशा व्यक्तींच्या/बालकाच्या पालकाकडून घेतली जावी.
3. मानसिक आजाराने पिडीत असलेल्या व्यक्तीकडून, त्यांच्या सध्याच्या स्थितीबाबत नेमून दिलेल्या अधिका-याने दिलेल्या माहितीच्या आधारावर एच.आय.व्ही चाचणीसाठी संमती घेण्यात यावी अथवा अशा व्यक्तींच्या काळजीची जबाबदारी स्विकारलेल्या व्यक्तीकडून एच.आय.व्ही चाचणी करण्यापूर्वी संमती घेण्यात यावी.
4. बेशुध्दावस्थेतील रुग्णांच्या बाबतीत, उपचारांच्या दृष्टीने एच.आय.व्ही. संसर्गाचे निदान करण्याची आवश्यकता असल्यास, या संबंधीची लिखित संमती रुग्णाचे पालक, पती/ पत्नी जवळचे नातेवाईक यांच्यापैकी, जो त्यावेळी उपस्थित असेल त्याच्याकडून घेण्यात यावी. रुग्णांच्या नातेवाईकांपैकी कोणीही उपलब्ध नसल्यास, आणि उपचारांसाठी अशी चाचणी अत्यावश्यक असलयास, रुग्णावर उपचार करणा-या दोघा डॉक्टरांची याबाबतीची शिफारस /अनुमती घेऊनच ही चाचणी करण्यात यावी.
5. जर रुग्णास वैद्यकीय दृष्टया फायदेशीर ठरत असेल तर एच.आय.व्ही संसर्गाची स्थितीसहित इतर गोपनीय वैद्यकीय माहिती अनैच्छिक रित्या (Non Voluntary Disclosure) उघड करता येऊ शकते, किंवा रुग्णांच्या ओळखता येण्याजोग्या साथीदारास (Identifiable Partner) रुग्णाकडून एच.आय.व्ही संसर्गाचा संभाव्य लैंक्षणिक धोका असल्यास पण अशी गोपनीय माहिती उघड करता येऊ शकते. ही माहिती रुग्णांच्या उपचारात प्रत्यक्ष सहभाग असलेल्या अधिका-यापुढे उघड करण्यात यावी जर रुग्णांच्या जीवाला (आत्महत्येच्या विचारांचा) किंवा त्याच्या/तिच्या साथीदाराच्या/ पती/ पत्नीच्या जीवाला धोका असेल तरी दे खील ही माहिती उघड करता येऊ शकते.(Partner Notification)

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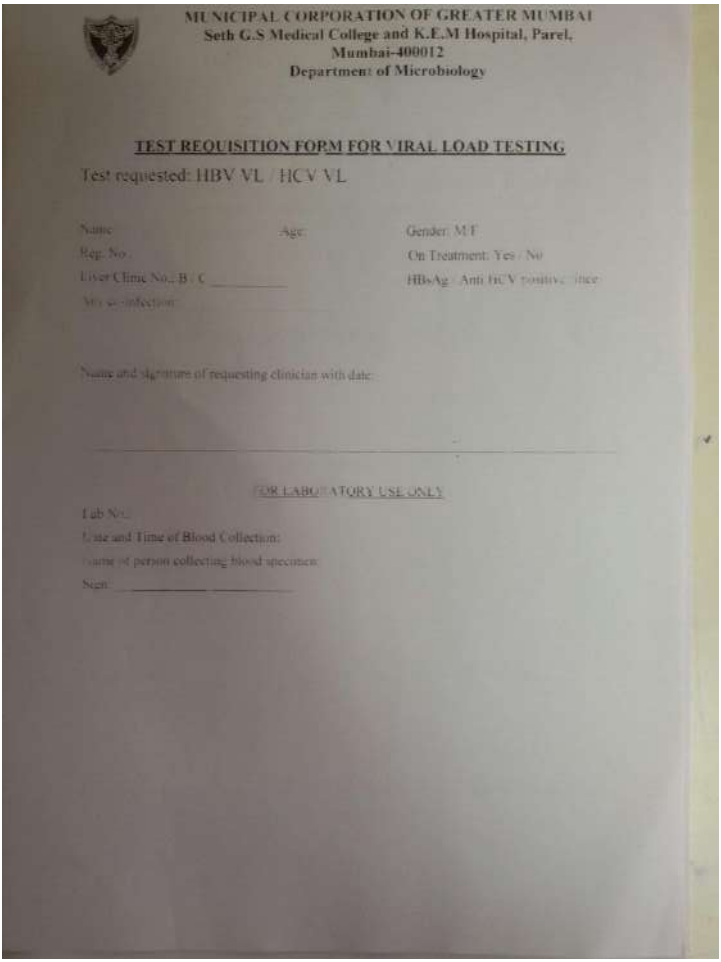
APPENDIX 4
COMMON TEST REQUISITION FORM
(Tests other than HIV antibody, CD4 count and viral loads)

LAB NO

MUNICIPAL CORPORATION OF GREATER MUMBAI
SETH G.S MEDICAL COLLEGE AND K.E.M HOSPITAL, PAREL, MUMBAI-400012
DEPARTMENT OF MICROBIOLOGY
TEST REQUISITION FORM

Nature of Specimen –	Patient details
Date of collection:	**Name :**
Time of collection:	**Age / Gender :**
Site of collection: **(where applicable)**	**Reg no:** **OPD / Ward** _____ **Unit** _____
Investigation required **(please tick any one only)**	**Diagnosis**
Clinical Bacteriology (7th floor) - Only Microscopy (MI) - MI , Aerobic culture and ABS - MI and Anaerobic culture - Stool (cholera)*	**Tick appropriate** - Community acquired / - Hospital acquired ***Full address mandatory** **(Lepto/Dengue/Chik V/Cholera/Typhoid)**
Mycobacteriology (5th floor) - AFB smear - AFB culture	
Mycology (5th floor) - Microscopy - Culture - Others _____	
Parasitology (5th floor) - Stool – routine & microscopy - Stool – opportunistic parasites - Blood - malaria antigen - Other (please specify below) _____	**# Relevant clinical information** Fever : yes / no Duration : Joint pain : yes / no Rash: yes / no Flood water contact yes / no Any other : _____
Serology (5th floor) - Rheumatoid Arthritis (RA) factor test - Anti Streptolysin O test - Widal test - VDRL test - Antibody – Leptospires*# - Antibody –Dengue*# - Antigen – Dengue NS1 - Antibody – Chikungunya*#	**Name and Signature of requesting clinician with date** _____
Virology and Immunology(5th floor) - Antibody – HIV - Antibody – Hepatitis C virus - Hepatitis B surface antigen - CD4 count	**For laboratory use only** Date specimen received : _____ Time received : ; _____
Molecular Diagnostics (7th floor) - HIV viral load	Name & Sign of receiver : _____
Any other investigation (not listed above)	

APPENDIX 5



MUNICIPAL CORPORATION OF GREATER MUMBAI
Seth G.S Medical College and K.E.M Hospital, Parel,
Mumbai-400012
Department of Microbiology

TEST REQUISITION FORM FOR VIRAL LOAD TESTING

Test requested: HBV VL / HCV VL

Name: Age: Gender: M/F

Reg. No.: On Treatment: Yes / No

Liver Clinic No.: B / C _____ HBsAg / Anti HCV positive since:

Any co-infection:

Name and signature of requesting clinician with date:

FOR LABORATORY USE ONLY

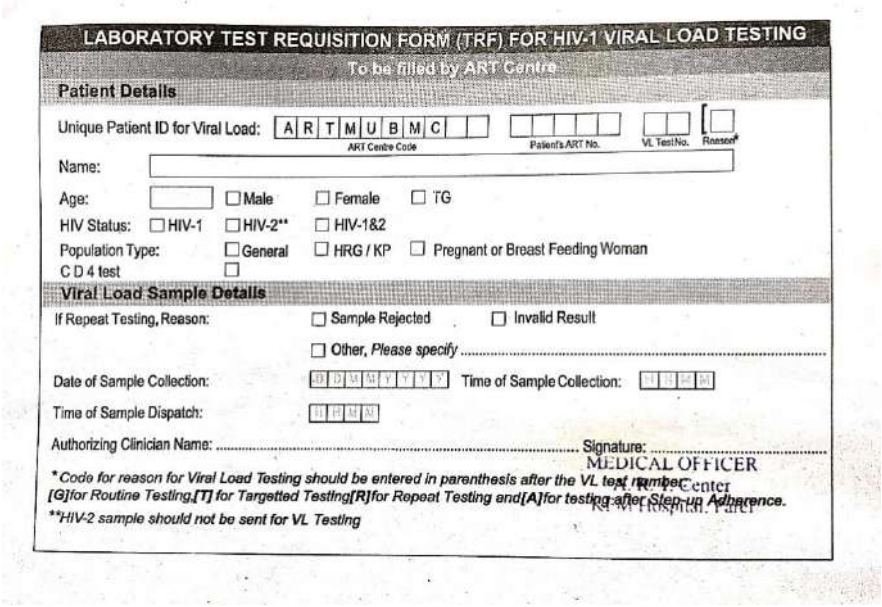
Lab No.:

Date and Time of Blood Collection:

Name of person collecting blood specimen:

Sign: _____

Appendix 6
Requisition form for HIV Viral Load and/or CD4 count estimation



LABORATORY TEST REQUISITION FORM (TRF) FOR HIV-1 VIRAL LOAD TESTING

To be filled by ART Centre

Patient Details

Unique Patient ID for Viral Load: A R T M U B M C | ART Centre Code | Patient's ART No. | VL Test No. | Reason*

Name:

Age: ☐ Male ☐ Female ☐ TG

HIV Status: ☐ HIV-1 ☐ HIV-2** ☐ HIV-1&2

Population Type: ☐ General ☐ HRG / KP ☐ Pregnant or Breast Feeding Woman

C D 4 test ☐

Viral Load Sample Details

If Repeat Testing, Reason: ☐ Sample Rejected ☐ Invalid Result

☐ Other, *Please specify* .....

Date of Sample Collection: D D M M Y Y Y Y Time of Sample Collection: H H M M

Time of Sample Dispatch: H H M M

Authorizing Clinician Name: ..... Signature: .....

MEDICAL OFFICER

**Code for reason for Viral Load Testing should be entered in parenthesis after the VL test number.*
[G]for Routine Testing,[T] for Targetted Testing[R]for Repeat Testing and[A]for testing after Step-up Adherence.
***HIV-2 sample should not be sent for VL Testing*

APPENDIX 7
LABORATORY FORM FOR SPUTUM EXAMINATION

Annexure 15A

NTEP Request Form for examination of biological specimen for TB
(Required for Diagnosis of TB, Drug susceptibility Testing and follow up)

Patient Information

Patient name		**Age (in yrs):**____	**Gender:** ☐M ☐F ☐TG
Patient mobile no. or other contact no.		**Specimen collection date** (DD/MM/YY) _____	☐ Sputum ☐ Other (specify)_____
Aadhaar no.(if available)		**HIV Status:** ☐Reactive ☐Non-Reactive ☐Unknown	
Patient address with landmark		**Key populations**: ☐Contact of known TB Patient ☐Contact of known DR TB Patient ☐Diabetes ☐Tobacco ☐Prison ☐Miner ☐Migrant ☐Refugee ☐ Urban slum ☐Health-care worker ☐Other(specify)_____	

Name and Type of referring facility (PHI/DMC/TU/ DTC/ICTC/ART/Medical College/DR-TB Centre/RBSK/Private Others, specify)_____ Type of patient: ☐ Public sector ☐ Private sector
Episode ID: _____
Health Establishment ID (NIKSHAY): _ _ _ _
State: _____ **District:** _____ **Tuberculosis Unit (TU):** _____

Reason for Testing

Diagnosis and follow up of TB

Diagnosis of TB (for presumptive TB)	Follow up (Smear and culture)
H/O anti TB Rx for >1 month: ☐ Yes ☐ No	Reason: ☐ End IP ☐ End CP
☐ TB symptomatic ☐ Any abnormality in X-ray ☐ Repeat Exam ☐ Presumptive NTM Predominant symptom _____ Duration: ___days	Post treatment: ☐ 6m ☐ 12m ☐ 18m ☐ 24m

Diagnosis and follow up Drug-resistant TB

Diagnosis of DR TB (DRT/ DST)		Follow up (Smear & culture)
Presumptive MDR TB	☐ New ☐ Previously treated ☐ At TB diagnosis ☐ Follow up Sm+ve DS TB	Treatment follow up month: ____ Type of case: ☐ H mono/poly TB ☐ MDR/RR TB ☐ XDR TB
	☐ Presumptive H mono/poly	Regimen Type: ☐ All oral H mono/poly TB regimen ☐ Shorter MDR TB regimen ☐ All oral longer regimen ☐ Any other regimen
Presumptive XDR TB	☐ MDR/RR TB at Diagnosis ☐ Failure of MDR/RR TB regimen ☐ Recurrent case of second line treatment	Regimen composition: ☐ Lfx ☐ Mfx[h] ☐ Bdq ☐ Lzd ☐ Cfz ☐ Cs ☐ Z ☐ E ☐ Eto ☐ Dlm ☐ Am ☐ Km ☐ Cm ☐

Test requested:
☐ Microscopy ☐TST ☐IGRA ☐Chest X-ray ☐Cytopathology ☐Histopathology ☐ CBNAAT ☐ TruNAAT
☐ Culture ☐DST ☐FL-LPA ☐SL-LPA ☐Gene Sequencing ☐Other (Please Specify) _____
Requested by (Contact No. & Designation and Signature): _____
Contact Number: _____ Email ID: _____

Results:

Microscopy (☐ ZN ☐ Florescent) Test ID: _____

	Lab Sr. No	Visual appearance	Result				
			Negative	Scanty	1+	2+	3+
Sample A		S M B					
Sample B		S M B					

Date tested: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ (Name and Signature)

APPENDIX 8
LABORATORY FORM FOR XPERT MTB/RIF ASSAY TEST
(pg 1/2 Front)

Annexure 15A

NTEP Request Form for examination of biological specimen for TB
(Required for Diagnosis of TB, Drug susceptibility Testing and follow up)

Patient Information

Patient name		**Age (in yrs):**____	**Gender:** ☐M ☐F ☐TG
Patient mobile no. or other contact no.		**Specimen collection date** (DD/MM/YY) _____	☐ Sputum ☐ Other (specify)_____
Aadhaar no.(if available)		**HIV Status:** ☐Reactive ☐Non-Reactive ☐Unknown	
Patient address with landmark		**Key populations**: ☐Contact of known TB Patient ☐Contact of known DR TB Patient ☐Diabetes ☐Tobacco ☐Prison ☐Miner ☐Migrant ☐Refugee ☐ Urban slum ☐Health-care worker ☐Other(specify)_____	

Name and Type of referring facility (PHI/DMC/TU/ DTC/ICTC/ART/Medical College/DR-TB Centre/RBSK/Private Others, specify): _____ Type of patient: ☐ Public sector ☐ Private sector
Episode ID: _____
Health Establishment ID (NIKSHAY): _ _ _ _
State: **District:** **Tuberculosis Unit (TU):** _____

Reason for Testing

Diagnosis and follow up of TB

Diagnosis of TB (for presumptive TB)	Follow up (Smear and culture)
H/O anti TB Rx for >1 month: ☐ Yes ☐ No	Reason: ☐ End IP ☐ End CP
☐ TB symptomatic ☐ Any abnormality in X-ray ☐ Repeat Exam ☐ Presumptive NTM — Predominant symptom _____ Duration: ___days	Post treatment: ☐ 6m ☐ 12m ☐ 18m ☐ 24m

Diagnosis and follow up Drug-resistant TB

Diagnosis of DR TB (DRT/ DST)		Follow up (Smear & culture)
Presumptive MDR TB	☐ New ☐ Previously treated ☐ At TB diagnosis ☐ Follow up Sm+ve DS TB	Treatment follow up month: ____ Type of case: ☐ H mono/poly TB ☐ MDR/RR TB ☐ XDR TB
	☐ Presumptive H mono/poly	Regimen Type: ☐ All oral H mono/poly TB regimen ☐ Shorter MDR TB regimen ☐ All oral longer regimen ☐ Any other regimen
Presumptive XDR TB	☐ MDR/RR TB at Diagnosis ☐ Failure of MDR/RR TB regimen ☐ Recurrent case of second line treatment	Regimen composition: ☐ Lfx ☐ Mfx[h] ☐ Bdq ☐ Lzd ☐ Cfz ☐ Cs ☐ Z ☐ E ☐ Eto ☐ Dlm ☐ Am ☐ Km ☐ Cm ☐

Test requested:
☐ Microscopy ☐TST ☐IGRA ☐Chest X-ray ☐Cytopathology ☐Histopathology ☐ CBNAAT ☐ TruNAAT ☐ Culture ☐DST ☐FL-LPA ☐SL-LPA ☐Gene Sequencing ☐Other (Please Specify) _____
Requested by (Contact No. & Designation and Signature): _____
Contact Number:_____ Email ID:_____

Results:

Microscopy (☐ ZN ☐ Florescent) Test ID: _____

	Lab Sr. No	Visual appearance	Result				
			Negative	Scanty	1+	2+	3+
Sample A		S M B					
Sample B		S M B					

Date tested: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ (Name and Signature)

APPENDIX 8 (contd)
LABORATORY FORM FOR XPERT MTB/RIF ASSAY TEST
(pg 2/2 Back)

Date of specimen received: _____

Nucleic Acid Amplification Test (NAAT) Lab serial _____ Test ID: _____

Type of test	☐ CBNAAT	☐ TrueNat
Sample	☐ A	☐ B
M. Tuberculosis	☐ Detected	☐ Not Detected ☐ N/A
Rif Resistance	☐ Detected	☐ Not Detected ☐ Indeterminate ☐ N/A
Test	☐ No Result ☐ Invalid	☐ Error – Error Code_____ (Please arrange for fresh sample)

Date tested: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**

Culture (☐ LJ ☐ LC) Test ID: _____

Lab Sr. No	Negative	Positive	NTM (write species)	Contamination

Date Result: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**

First line LPA Lab serial _____ Test ID: _____

☐ Direct ☐ Indirect ☐ Valid ☐ Invalid ☐ MTB detected ☐MTB not detected

Drug	Resistant detected	Final Interpretation	Remark
Rifampicin (R)	☐ Yes ☐ Inferred ☐ No	If yes or inferred, R should not be given	
Isoniazid (Kat G)	☐ Yes ☐ Inferred ☐ No	If yes or inferred, H(h) should not be given	
Isoniazid (Inh A)	☐ Yes ☐ Inferred ☐ No	If yes or inferred, H(h) can be considered & Eto should not be given	

Date Result: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**

Second line LPA Lab serial _____ Test ID: _____

☐ Direct ☐ Indirect ☐ Valid ☐ Invalid ☐ MTB detected ☐MTB not detected

Drug	Resistant detected	Final interpretation	Remark
Levofloxacin	☐ Yes ☐ Inferred ☐ No	If yes or inferred, Lfx should not be given. Mfx (h) can be considered.	
Moxifloxacin (h)	☐ Yes ☐ No	If yes, Lfx & Mfx (h) should not be given	
Amikacin	☐ Yes ☐ Inferred ☐ No	If yes or inferred, Am should not be given	
Kanamycin	☐ Yes ☐ Inferred ☐ No	If yes or inferred, Km should not be given	
Capreomycin	☐ Yes ☐ Inferred ☐ No	If yes or inferred, Cm should not be given	

Date Result: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**

Drug Susceptibility Test (DST) results Test ID: _____

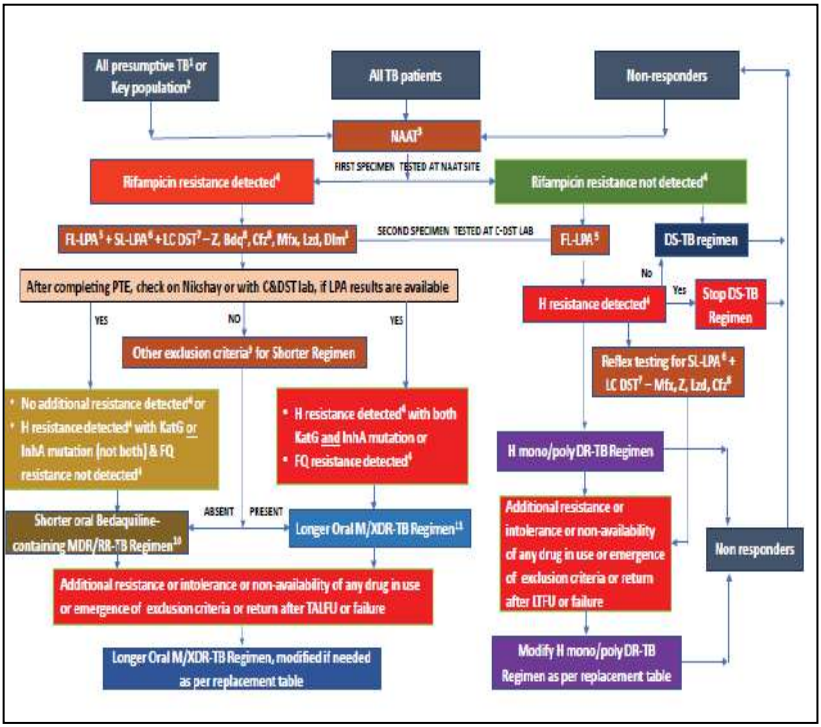
Lab Sr.No	1st line drugs					SLI				FQ				Other										
	R	H (0.1)	H (0.4)	Z	E	S	Km	Cm	Am	Lfx	Mfx (0.5)	Mfx (1.0)	Mfx (2.0)	PAS	Lzd	Cfz	Bdq	Dlm	Eto	Cs	Clr	Azi		

Date Result: _____ **Date Reported:** _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**
R: Resistant; S: Susceptible; C: Contaminated; – Not done

Other tests for TB diagnosis Test ID: _____

Test (Please Specify): _____
Result: _____
Date reported: _____ **Reported by:** _____
Laboratory Name: _____ **(Name and Signature)**

Appendix 9
Diagnostic algorithm for TB (PMDT 2021 guidelines)



ICMR Specimen Referral Form for COVID-19 (SARS-CoV2)

INTRODUCTION

This form is for collection centres/ labs to enter details of the samples being tested for Covid-19. It is mandatory to fill this form for each and every sample being tested. It is essential that the collection centres/ labs exercise caution to ensure that correct information is captured in the form.

INSTRUCTIONS

- Inform the local / district / state health authorities, especially surveillance officer for further guidance
- Seek guidance on requirements for the clinical specimen collection and transport from nodal officer
- This form may be filled in and shared with the IDSP and forwarded to a lab where testing is planned
- Fields marked with asterisk (*) are mandatory

SECTION A – PATIENT DETAILS

A.1 TEST INITIATION DETAILS

*Sample collected first time: Yes ☐ No ☐

If No, Patient ID: .....

A.2 PERSONAL DETAILS

*Patient Name: ..... Father's Name .....

*Age: ..... Years/Months/ Days (If age <1 yr, pls. tick months/ days checkbox)

* Gender: Male ☐ Female ☐ Transgender ☐

*Occupation: Health Care Worker ☐ Police ☐ Sanitation ☐ Security Guards ☐ Others ☐

*Mobile Number: ☐☐☐☐☐☐☐☐☐☐ Mobile Number belongs to: Patient ☐ Family ☐

*Nationality: .....

*Present patient address: ..... *Downloaded Aarogya Setu App: Yes ☐ No ☐

Pincode: ..... *Location: Urban ☐ Rural ☐ Tribal ☐ (Select either of the ones)

*District ..... *State : .....

(These fields to be filled for all patients including foreigners)

Aadhar No. (For Indians): .....

Passport No. (For Foreign Nationals): .....

*Received COVID-19 vaccine Yes ☐ No ☐

*CoWIN Beneficiary ID (If Available, else leave it Blank).....

*If yes type of vaccine (in drop down) Covaxin (Bharat Biotech) ☐ Covishield (Serum Institute of India) ☐

Sputnik V (The Gamaleya National Center) ☐ BNT162b2 (Pfizer-BioNTech) ☐ mRNA-1273 (Moderna) ☐

Ad26.COV2.5 (Johnson & Johnson) ☐ Covovax (Serum Institute of India) ☐ ZyCoV-D (Zydus Cadila) ☐

NVX-CoV2373 (Novavax) ☐ Covilo (SinoPharm) ☐ CoronaVac (Sinovac) ☐

*Date of Dose 1 ----/----/---------- *Dose 2 received? – Yes/ No (Mandatory) If yes, Date of Dose 2 ----/----/---------- (Mandatory)

A.3 SPECIMEN INFORMATION FROM REFERRING AGENCY

*Specimen type: Throat Swab ☐ Nasal Swab ☐ Bronchoalveolar lavage ☐ Endotracheal Asprate ☐ Nasopharyngeal swab ☐

*Type of test RT-PCR ☐ Rapid Antigen Test (RAT) ☐

*Name of kit used: .....

*Collection date: --/--/----

*Sample ID (Label) .....

Symptomatic ☐ Asymptomatic ☐

Contact of a lab confirmed case: Yes ☐ No ☐

If, RT-PCR test, name of lab where sample is sent for testing (Drop down – list of RT-PCR/ TrueNat/ CBNAAT labs)

* Mode of Transport used to visit testing facility ☐ Public – In drop down menu – Bus, Metro, Train, Cab, Auto, Ambulance
☐ Private – In drop down menu – Car, Scooty, Bike, Bicycle, Walk
☐ Not Applicable

Please Note - Hospital form is required for the patients visiting OPD, IPD and Emergency and Community form is required for patients under containment zone/ Non-containment area/ Point of entry/ Testing on demand

1

***A.3.1 For Community**

Sample collected from (In Dropdown) - **Containment Zone/Non-containment area/Point of entry (Select either of the ones)**

Cat 1: All symptomatic (ILI symptoms) cases
Cat 2: All asymptomatic high-risk individuals (Any individual who falls under Section B2)
Cat 3: All symptomatic (ILI symptoms) individuals with history of international travel in the last 14 days
Cat 4: All individuals who wish to get themselves tested

A.3.2 For Hospital

Cat 1: All patients of Severe Acute Respiratory Infection (SARI)
Cat 2: All symptomatic (ILI symptoms) patients presenting in a healthcare setting
Cat 3: Asymptomatic high-risk patients who are hospitalized or seeking immediate hospitalization
Cat 4: Asymptomatic patients undergoing surgical / non-surgical invasive procedures (not to be tested more than once a week during hospital stay).
Cat 5: All pregnant women in/near labour who are hospitalized for delivery
Cat 6: All symptomatic neonates presenting with acute respiratory / sepsis like illness
Cat 7: Patients presenting with atypical manifestations [stroke, encephalitis, pulmonary embolism, acute coronary symptoms, Guillain Barre syndrome, Multi-system Inflammatory Syndrome in Children (MIS-C), progressive gastrointestinal symptoms] based on the discretion of the treating physician
Cat 8: All individuals who wish to get themselves tested

**Fields marked with astertsk are mandatory to be filled*

Please Note: Section B1 and B2 need to be filled for both Community and Hospital settings. Section B3 needs to be filled only for Hospital settings

SECTION B- MEDICAL INFORMATION

B.1 CLINICAL SYMPTOMS AND SIGNS

Cough	☐	Loss of taste	☐
Sore Throat	☐	Diarrhoea	☐
Fever	☐	Breathlessness	☐
Loss of smell		Other symptoms, please specify:	

ate of onset of First Symptom(dd/mm/yy): ☐☐ ☐☐ ☐☐

B.2 PRE-EXISTING MEDICAL CONDITIONS

Diabetes	☐	Over weight/ Obesity	☐
Heart disease	☐	Hypertension	☐
Chronic Lung disease	☐	Cancer	☐
Chronic Kidney Disease	☐	Any other please specify:	

B.3 HOSPITALIZATION DETAILS

Hospitalized: Yes ☐ No ☐

Hospital State: ..... Hospital

District: .....

Hospitalization Date: ☐☐ ☐☐ ☐☐

Hospital Name: .....

TEST RESULT (To be filled by Covid-19 testing lab facility)

Date of sample receipt(dd/mm/yy)	Sample accepted/ Rejected	Date of Testing (dd/mm/yy)	Test result (Positive / Negative)	Repeat Sample required (Yes / No)	Sign of Authority (Lab in charge)

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Kindly send your suggestions if any to the office of
Professor and Head,
Department of Microbiology,
7th floor, MSB.