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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.**

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**AMENDMENT RECORD**

<b>S r N o</b>	<b>P g N o</b>	<b>Section/ Clause / Para / Line</b>	<b>Date of Amendm ent</b>	<b>Amendme nt made</b>	<b>Reason for amendme nt</b>	<b>Signatur e of person authorizi ng amendm ent</b>
1	2	Distributi on list	3.1.2022	Online availabilit y No physical print copies	Ease of use and larger access	
	15 - 18	Contact person for various divisions	3.1.2022	Transfer of faculty and reassignm ent 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.	Comple ness	
	73	TAT SARS- CoV-2 IgG antibody	3.1.2022	TAT for SARS- CoV-2 IgG antibody added	Inclusion	

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	<b>1</b>	<b>Release authorization</b>	<b>11.02.2023</b>	<b>Professor and Head</b>	<b>Super-annuation of former Professor and Head</b>	
	<b>15</b>	<b>Laboratory working hours</b>	<b>11.02.2023</b>	<b>COVID-19 RTPCR</b>	<b>As per new advisory from ICMR</b>	
	<b>16</b>	<b>Specimen acceptance timing</b>	<b>11.02.2023</b>	<b>COVID-19</b>	<b>Revised</b>	
	<b>17</b>	<b>Contact person</b>	<b>11.02.2023</b>	<b>Added Dr. Chaya Kumar</b>	<b>Joined new as Prof. and Head</b>	
	<b>20</b>	<b>Contact person</b>	<b>11.02.2023</b>	<b>Added Dr. Chaya Kumar</b>	<b>Joined new as Prof. and Head</b>	

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**List of Abbreviations**

<b><u>Abbreviations</u></b>	<b><u>Full Form</u></b>
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

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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

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## 1. **FOREWORD**

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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 7<sup>th</sup> 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**

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*'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**

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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)

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- 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).

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- 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.

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#### **4. LABORATORY WORKING HOURS**

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

Routine working hours – All divisions	Weekdays	9.00 a.m. to 4.00 p.m.
	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 except Sundays	Patients' samples (as per latest advisory) will be accepted till 5 pm except Sundays. Samples of COVID symptomatic will be accepted throughout the day including Sundays.

#### **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	Clinical Bacteriology	9.00 a.m. – 11.00 a.m.

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Sputum		
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	Patient samples will be accepted from 9.00 a.m till 5 pm. Samples of COVID symptomatic 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
<b>Clinical Bacteriology</b> 7th floor, MSB	Microscopy & Culture for aerobic bacteria and anaerobic bacteria Antimicrobial susceptibility test on clinically relevant aerobic bacteria <b>MIC – Vitek2</b> 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	
<b>Molecular Diagnosis</b> 5th floor, MSB	HIV viral load for patients referred from ART	Whole blood in EDTA evacuated tube	Dr Shashir Wanjare / Dr. Vaishali
	HBV viral load (for		

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Division / Location	Tests offered	Specimen type * and number where applicable	Contact Person with intercom number
	patients referred from GI OPD) HCV viral load (for patients referred from GI OPD)		Surase / Dr. Ranjana Thate / Dr. Chaya Kumar 7039 / 7552
<b>Molecular Diagnosis</b> (7 <sup>th</sup> 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
<b>Mycology</b> 5 <sup>th</sup> 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
<b>Mycobacteriology</b> 5 <sup>th</sup> floor, MSB	Microscopy (LED fluorescent microscopy)	Sputum** – at least 2 specimens of which one is early morning and the other is spot.  Gastric lavage – 3 specimens collected on 3 different days, Other specimens – One or more	Dr Swapna Kanade  7827

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Division / Location	Tests offered	Specimen type * and number where applicable	Contact Person with intercom number
	Culture - MGIT	At least 3 ml in case of non-tissue specimens	Dr Swapna Kanade  7827
	1 <sup>st</sup> Line, 2 <sup>nd</sup> line and Pyrazinamide DST - MGIT	Sputum (currently for followup patients as per NTEP guidelines)	
	<b>Xpert MTB/RIF ** assay<sup>#</sup></b> 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, 5 <sup>th</sup> floor CVTS building)	
<b>Parasitology</b> 5 <sup>th</sup> 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	
<b>Serology</b> 5 <sup>th</sup> floor, MSB	ASO Dengue – NS1 antigen (Rapid / ELISA) Dengue – IgG and IgM antibodies (Rapid /	Whole blood collected in clean, dry, plain test tube / red top evacuated tubes.	Dr Vijaya Torane  7984 / 7985

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Division / Location	Tests offered	Specimen type * and number where applicable	Contact Person with intercom number
	<p>ELISA) Leptospirosis – IgM Antibodies ( Rapid / ELISA) RF Widal RPR / V.D.R.L Chikungunya IgM Antibody</p> <p><b>Covid-19 IgG antibody Referral of specimens to PCR laboratory at Kasturba Hospital for Leptospirosis, Dengue</b></p> <p>pdmH1N1/2009</p>	<p>Serum</p> <p>PCR – 3-5 ml blood in purple cap (EDTA) evacuated tubes and transported in cold chain</p> <p>Collect sample (nasal/throat) using the nylon swab provided with VTM kit, place in VTM and transport in cold chain</p>	
	<b>COVID-19 RAT</b>	As provided in the kit. Usually NP swab with buffer solution	Dr. Priyanka Prasad

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Division / Location	Tests offered	Specimen type * and number where applicable	Contact Person with intercom number
<b>Virology and Immunology</b>  5 <sup>th</sup> floor, MSB –	ICTC <sup>@</sup>  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. Chaya Kumar / 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
<b>CLINICAL BACTERIOLOGY DIVISION</b>			
1	<b>Blood culture (conventional)</b> Aerobic culture & Antimicrobial susceptibility test	CRBSI, Enteric fever, Infection of prosthetic material ( implants), Infective endocarditis (IE), Meningitis, Osteomyelitis, Pneumonia, PUO,	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.

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
		Septicemia	
2	<b>Blood culture (Automated method BACTEC 9050)</b> 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	<b>Normally sterile body fluids</b> – culture C.S.F, Pleural, Pericardial, Peritoneal (Ascitic), Joint, Smear, Culture and Antimicrobial susceptibility test	Infection at respective sites	Negative microscopy or culture does not rule out disease. Larger volumes improve sensitivity.
4	<b>Throat swab from suspected diphtheria</b> 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	<b>Sputum</b> - Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired	Both sensitivity and specificity are considered $\leq 50\%$ unless expectorated sputum is purulent.
6	<b>Respiratory samples culture</b> (mini BAL, BAL, endotracheal aspirate) Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired Counts $\geq 10^4$ cfu/ml correlates better with disease though not always	Difficult to distinguish colonization from infection even with quantitative cultures. Clinical correlation essential.
7	<b>Miscellaneous</b>	Suspected	Used to rule in disease.

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
	(Pharyngeal swabs, Skin scraping ) Smear, Culture and Antimicrobial susceptibility test	streptococcal pharyngitis, Localised skin infections	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. Swabs need to be transported to lab immediately. A dried swab is detrimental to growth and can give false negative results.
8	<b>Ocular specimens</b> (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	<b>Pus</b> Smear, Culture and Antimicrobial susceptibility test	Localised skin or organ specific	Sensitivity – 70% Specificity - High
10	<b>Wound swab</b> Smear examination by microscopy	Bacterial cellulitis, gas gangrene	Microscopy and culture unreliable. Collect tissue material or purulent discharge whenever possible.
11	<b>Tissue</b> (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.

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
			Swabs collected without appropriate debridement will yield contamination / false negative result.
12	<b>Specimens from female genital tract</b> ( Vaginal /cervical swab, Urethral discharge, product of conception ) and urethral discharge Smear, Culture and Antimicrobial susceptibility test	Vaginitis, cervicitis, urethritis	Specimens from lower genital tract will be contaminated with normal flora and difficult to interpret.
13	<b>Stool Microscopy</b> – hanging drop	Diarrhoeas, purulent enterocolitis	A negative test for darting motility does not rule out cholera (sensitivity and specificity ~ 60%)
14	<b>Stool Culture &amp; Antimicrobial susceptibility test</b>	Diarrhoeas, dysentery, purulent enterocolitis	Necessary to process specimens immediately to prevent overgrowth by normal flora.
15	<b>Urine Smear, culture &amp; Antimicrobial susceptibility test</b>	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

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
			not exclude another site of serious infection. -Prior antibiotic therapy may lead to negative urine culture in patients with UTI. -Sterile pyuria may be due to causes other than non-fastidious aerobic bacteria.
<b>SEROLOGY DIVISION</b>			
16	<b>RF Test for rheumatoid factors</b>	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	<b>ASO test</b>	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

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
18	<b>RPR / VDRL Test</b>	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 -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	<b>Widal Test</b>	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	<b>Lepto IgM rapid</b>	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

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
			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.
21	<b>Lepto IgM ELISA</b>	Qualitative detection of IgM class of antibodies against Leptospira by ELISA method.	Same as above
22	<b>Dengue NS1 – Rapid</b> (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	<b>Dengue NS1 – ELISA</b> (In a clinically compatible case, demonstration of NS1 antigen by ELISA is considered confirmatory)	Same as above	Same as above
24	<b>Dengue IgG/IgM Rapid</b> (As per notification received from MoHFW, GoI, a positive result by rapid test will be	Qualitative detection of IgG or IgM class of antibodies against dengue virus in human serum/ plasma by rapid immunochromatography method	- Not as specific or sensitive as ELISA -All positive results should always be correlated clinically -Intensity of test line depends on the stage

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
	<b>considered probable due to its poor sensitivity and specificity)</b>		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	<b>Dengue IgM ELISA (In a clinically compatible case, demonstration of Dengue IgM antibody by ELISA is considered confirmatory)</b>	Same as above	Same as above
26	<b>Chikungunya Antibody – ELISA (as per availability of kits)</b>	Qualitative detection of IgM class of antibodies against Chikungunya virus by ELISA method.	All positive results should be correlated clinically
27	<b>SARS CoV-2 IgG ELISA</b>	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	<b>COVID-19 RAT</b>	Symptomatic (cough, fever, sore throat, loss of taste and/or smell, breathlessness and/or other respiratory symptoms) individuals. At-risk contacts of laboratory confirmed	The sensitivity of the test being lower than RT-PCR, all RAT negative symptomatic individuals should be confirmed by RT-PCR. The test has to be

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
		cases. [At-risk contacts are elderly (>60yr) and individuals with co-morbidity such as diabetes, hypertension, chronic lung or kidney disease, malignancy, obesity etc].	processed within 30 minutes of collection.

**MYCOBACTERIOLOGY DIVISION**  
( Also refer to Appendix 9 )

29	<b>Microscopy</b>	Clinical suspicion of PTB / EPTB	Sensitivity low ( 10 <sup>5</sup> orgs/ml)
30	<b>Culture</b>	All EPTB cases and suspected MDRTB cases as per recent PMDT guidelines	Solid culture – 4 / 6 weeks for report Liquid culture - contamination
31	<b>XpertMTB/RIF assay</b>	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	<b>Stool / other specimens - Microscopy</b>	Suspected parasitic infection in immunocompetent / immunocompromised patients	For detecting trophozoites, fresh stool specimen essential to be examined within the hour of collection. A negative result on a single stool specimen does not rule out parasitic presence.
33	<b>Blood – RDT</b>	Clinically suspected	- Detection limit is

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
	<b>malarial antigen</b>	malaria cases	usually 200 parasites / $\mu$ l. 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 <i>P. falciparum</i> and the other is found in all 4 human species of malaria. Thus, microscopy is needed to determine the species of malaria other than <i>P.falciparum</i> .
<b>MYCOLOGY DIVISION</b>			
34	<b>Any specimen – Microscopy(KOH)</b>	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
35	<b>Microscopy – India ink</b>	Suspected cryptococcal infection	-The diagnosis of <i>C. neoformans</i> by India ink staining should be considered a presumptive result - Culture, biochemical

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			and serological testing is recommended for final identification. Some strains of <i>C. neoformans</i> , as well as other cryptococci may not produce discernible capsule
36	<b>Culture</b>	Suspected superficial or deep fungal infection	-Longer time required for growth of different fungi -Contamination by saprophytic fungi

**VIROLOGY AND IMMUNOLOGY DIVISION**

37	<b>HIV Antibody tests (Rapid)</b>	<p>-Patients who present with symptoms suggestive of HIV infection. Examples pneumonia, TB or persistent diarrhoea.</p> <p>-Patients with conditions that could be associated with HIV such as STI/RTI.</p> <p>-Prevention of parent (mother) to child transmission - pregnant women who register at ANCs. These also include pregnant</p>	<p>-False Negative result : in window period &amp; terminal stage of HIV disease</p> <p>-False positive result: autoimmune disease, multiple blood transfusion, pregnancy etc.</p>
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Sr.no.	Specimen / test performed	Indications (major)	Limitations
		women who directly come in labour without any antenatal check-up	
38	<b>HBsAg ELISA</b>	<ul style="list-style-type: none"> <li>Signs/symptoms suggestive of hepatitis</li> <li>H/o exposure</li> </ul>	-False Negative : during incubation period  -False positive: due to presence of other antigens or elevated levels of Rheumatoid factor
39	<b>Anti HCV ELISA</b>	<ul style="list-style-type: none"> <li>Signs/symptom suggestive of hepatitis</li> <li>H/o exposure</li> </ul>	-False Negative: in window period  -False positive: elevated levels of Rheumatoid factor  - Cannot differentiate recent from past infection
40	<b>RPR test</b>	<ul style="list-style-type: none"> <li>Direct walk in patients with high risk behavior</li> <li>Patients referred by the STI counselor</li> </ul>	-See page 22 above
41	<b>CD4 count</b>	<ul style="list-style-type: none"> <li>HIV positive patients referred from the ART centre</li> </ul>	-Nonspecific marker which can be affected by many other conditions

**MOLECULAR DIAGNOSIS**

42	<b>HIV viral load</b>	Monitoring response to	The detection limit
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Sr.no.	Specimen / test performed	Indications (major)	Limitations
		treatment	(sensitivity) varies between kits. The current test has a detection limit of 150 RNA copies / ml/
43	<b>HBV viral load</b>	Initiate treatment and monitor response to therapy	Limit of detection 6 IU/ml
44	<b>HCV viral load</b>		Limit of detection 9 IU/ml
45	<b>COVID-19 RT-PCR</b>	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**

46	<b>Lepto PCR</b>	Suspected leptospirosis, 1 <sup>st</sup> 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
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Sr.no.	Specimen / test performed	Indications (major)	Limitations
			same day of collection. Follow triple packaging while transporting. Transport in cold chain.
47	<b>Dengue PCR</b>	Suspected Dengue, 1 <sup>st</sup> week, NS1 Ag and IgM Ab negative	Same as above. Does not speciate.
48	<b>Throat / nasal swab for H1N1 influenza</b>	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

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- (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.

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- (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.

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### **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<sup>th</sup> 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 -**
  - 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.

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- 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.

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- 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

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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.

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- 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

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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.

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- 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.

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- 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

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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.

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## **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 aseptic 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)

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- 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

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- 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

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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 (1½–2 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,

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- 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 pre-labeled 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 pre-labeled 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

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- 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 7<sup>th</sup> floor, 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

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container provided by DOTS centre (5<sup>th</sup> 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.

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### **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.

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- 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, 5<sup>th</sup> floor, CVTS building.**

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## **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.

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- 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).

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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**

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- 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.

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- **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

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- 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 mortar 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**

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- 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**

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- 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<sup>nd</sup> floor, main hospital building) for PEP drugs

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- 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.

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## **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.

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## **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.

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- 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

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- 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

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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.

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### **31. COMPLAINTS**

For any complaints pertaining to any of the services offered, a note may be 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. 7<sup>th</sup> edition. Philadelphia: Wolters Kluwer Health; 2017
- Tille P. Bailey & Scott's Diagnostic Microbiology. 14<sup>th</sup> 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](http://www.searo.who.int/EN/Section10/Section17/Section53/Section482_1779.htm)
- GOI, MoHFW, No 7-165/2016/NVBDCP/DEN – Dated 9<sup>th</sup> June 2016
- Icmr.gov.in for all COVID-19 related documents.

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**APPENDIX 1**  
**Tests offered and their TAT (Next page)**

<b>Division</b>	<b>Test Offered</b>	<b>Volume</b>	<b>TAT After specimen receipt</b>
<b>Virology and Immunology Division</b>	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

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	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.
<p style="text-align: center;"><b>Clinical Bacteriology</b></p> <p>The container for collection should be clean, sterile and screw capped or plugged and appropriately labelled.</p>	Microscopy –  Gram’s stain, Albert’s stain	<b>1.0 ml</b> <b>Critical specimens –</b> <b>CSF, Tissue / swab for gas gangrene,</b> <b>Tissue / swab for Diphtheria, Pancreatic fluid, Brain abscess,</b> <b>Ocular specimens</b>	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 ( such as operation theatres) and swabs	24 hrs. for aerobic bacteria

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		from environmental and clinical contact surfaces as appropriate	72 hrs. for sporing anaerobes  5 days – 2 weeks to rule out fungal contamination
<b>Molecular Diagnosis</b>	COVID-19 RT-PCR	NP swab + OP swab / Nasal + throat swab / Nasal swab / Sputum / BAL	12 – 24 hrs from receipt of specimen
	Zika virus PCR	Blood	72 hrs
<b>Mycology</b>	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
<b>Mycobacteriology</b>	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.
<b>Parasitology</b> 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	5 ml / 1 gm of any	4 working hrs

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<b>Serology</b>	protozoon parasites	specimen	
	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		
Dengue and Lepto PCR	6 ml blood collected in EDTA evacuated tube	Result from Mol Diagnostic Lab – Kasturba	
pH1N1 PCR	Throat / Nasal swab in VTM		
	COVID-19 RAT	NP swab collected in buffer provided with kit	30 minutes from receipt
<b>Emergency Laboratory</b>	Critical specimens / critically ill patients <b>Microscopy</b> Gram's stain Albert's stain Indi Ink for Cryptococcus Stool-Hanging Drop <b>Culture – inoculation only</b>	1.0 ml	1 hr for critical specimens  2 hrs for others
	Malaria - RDT	3.0 ml (whole blood/serum)	2 hrs

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	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
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**APPENDIX 2**

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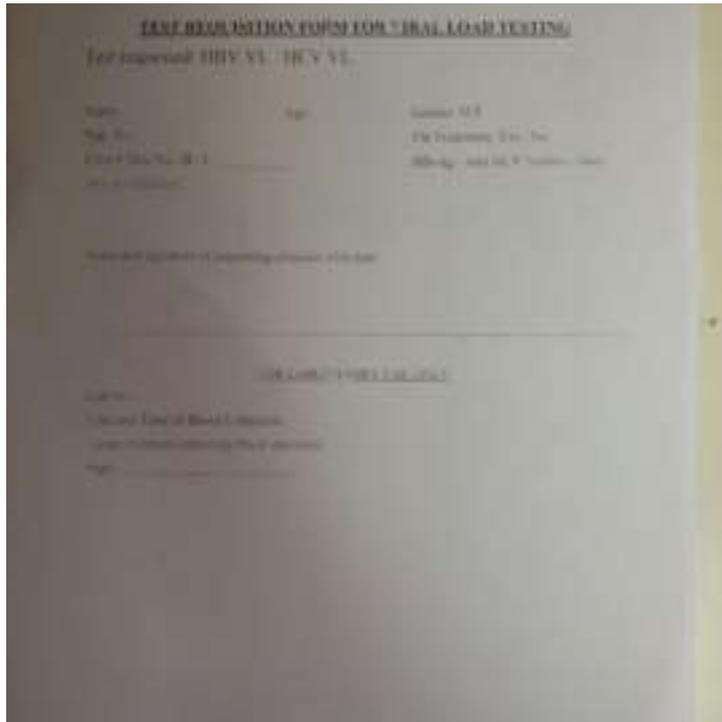
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**APPENDIX 4**  
**COMMON TEST REQUISITION FORM**  
(Tests other than HIV antibody, CD4 count and viral loads)

MUNICIPAL CORPORATION OF GREATER MUMBAI SETH G.S MEDICAL COLLEGE AND K.E.M HOSPITAL, PAREL, MUMBAI-400012 DEPARTMENT OF MICROBIOLOGY <b>TEST REQUISITION FORM</b>		IARMO
<b>Nature of Specimen –</b>	<b>Patient details</b>	
<b>Date of collection:</b>	<b>Name :</b> _____	
<b>Time of collection:</b>	<b>Age - Gender :</b> _____	
<b>Site of collection: (where applicable)</b>	<b>Reg no:</b> _____	
	<b>OPD / Ward _____ Unit _____</b>	
<b>Investigation required (please tick any one only)</b>	<b>Diagnosis</b>	
<b>Clinical Bacteriology (7th floor)</b> - Only Microscopy (MI) - MI + Aerobic culture and ABS - MI and Anaerobic culture - Stool (colored)*	Tick appropriate: - Community acquired / - Hospital acquired  * Full address mandatory (Lepto/Dengue/Chik V/Cholera/Typhoid)	
<b>Mycobacteriology (5<sup>th</sup> floor)</b> - AFB smear - AFB culture	_____ _____	
<b>Mycology (5<sup>th</sup> floor)</b> - Microscopy - Culture - Others _____	_____ _____	
<b>Parasitology (5<sup>th</sup> floor)</b> - Stool - routine & microscopy - Stool - opportunistic parasites - Blood - malarial 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: _____	
<b>Serology (5<sup>th</sup> floor)</b> - Rheumatoid Arthritis (RA) factor test - Anti Streptococcal O test - Widal test - VDRL test - Antibody - Leptospire** - Antibody - Dengue** - Antigen - Dengue NS1 - Antibody - Chikungunya**	<b>Name and Signature of requesting clinician with date</b>  _____	
<b>Virology and Immunology (5<sup>th</sup> floor)</b> - Antibody - HIV - Antibody - Hepatitis C virus - Hepatitis B surface antigen - CD4 count	<b>For laboratory use only</b>	
<b>Molecular Diagnostics (7<sup>th</sup> floor)</b> - HIV viral load	Date specimen received: _____	
	Time received: _____	
<b>Any other investigation (not listed above)</b>	Name & Sign of receiver: _____	

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**APPENDIX 5**



**Appendix 6**

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

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Appendix 8A

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

<b>Patient Information</b>		
Patient name	Age (in yrs):	Gender: <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/> OTH
Patient mobile no. or other contact no.	Specimen collection date (month):	<input type="checkbox"/> Sputum <input type="checkbox"/> Other (specify):
Aadhaar no. (if exists)		
Patient address with landmark	HIV Status: <input type="checkbox"/> Negative <input type="checkbox"/> Non-Response <input type="checkbox"/> Unknown Key populations: <input type="checkbox"/> Contact of known TB Patient <input type="checkbox"/> Contact of known DR TB Patient <input type="checkbox"/> Sex worker <input type="checkbox"/> Prison <input type="checkbox"/> Migrant <input type="checkbox"/> Refugee <input type="checkbox"/> Urban slum <input type="checkbox"/> Health-care worker <input type="checkbox"/> Other (specify):	

Name and Type of referring facility (HOSPITAL/DTCT/CDMT/ Medical College/ TB Centre/ RSC/ Private/ Other, specify): Health Establishment ID (NHSAY): State: District: Tuberculosis Unit (TU):	Type of patient: <input type="checkbox"/> Public sector <input type="checkbox"/> Private sector Episode ID:
---	--

<b>Reason for Testing</b>	
<b>Diagnosis and follow up of TB</b>	
Diagnosis of TB (for presumptive TB) HIO anti TB Rx for >1 month: <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> TB asymptomatic <input type="checkbox"/> Any abnormality in X-ray <input type="checkbox"/> Repeat Exam <input type="checkbox"/> Presumptive/NTM	Follow up (Smear and culture) Reason: <input type="checkbox"/> End IP <input type="checkbox"/> End OP Post treatment: <input type="checkbox"/> 6m <input type="checkbox"/> 12m <input type="checkbox"/> 18m <input type="checkbox"/> 24m Predominant symptom: Duration: days

<b>Diagnosis and follow up Drug-resistant TB</b>	
Diagnosis of DR TB (DRT/ DST) <input type="checkbox"/> New <input type="checkbox"/> Previously treated Presumptive MDR TB <input type="checkbox"/> AI TB diagnosis <input type="checkbox"/> Follow up Sm+ve DR TB <input type="checkbox"/> Presumptive H monotherapy Presumptive XDR TB <input type="checkbox"/> MDR/RR TB at Diagnosis <input type="checkbox"/> Failure of MDR/RR TB regimen <input type="checkbox"/> Recurred case of second line treatment	Follow up (Smear & culture) Treatment follow up month: Type of smear: <input type="checkbox"/> H monotherapy TB <input type="checkbox"/> MDR TB <input type="checkbox"/> XDR TB Regimen Type: <input type="checkbox"/> All oral H monotherapy TB regimen <input type="checkbox"/> Single MDR TB regimen <input type="checkbox"/> All oral longer regimen <input type="checkbox"/> Any other regimen Regimen composition: <input type="checkbox"/> Lx <input type="checkbox"/> Mx <input type="checkbox"/> Bx <input type="checkbox"/> Sx <input type="checkbox"/> Cx <input type="checkbox"/> Ds <input type="checkbox"/> E <input type="checkbox"/> F <input type="checkbox"/> Gx <input type="checkbox"/> Hx <input type="checkbox"/> Ix <input type="checkbox"/> Jx <input type="checkbox"/> Kx

Test requested: <input type="checkbox"/> Microscopy (TST) <input type="checkbox"/> DNA <input type="checkbox"/> Chest X-ray <input type="checkbox"/> Cytopathology <input type="checkbox"/> Histopathology <input type="checkbox"/> CRNAAT <input type="checkbox"/> TruNAAT <input type="checkbox"/> Culture <input type="checkbox"/> DST <input type="checkbox"/> LPA <input type="checkbox"/> SL-LPA <input type="checkbox"/> Gene Sequencing <input type="checkbox"/> Other (Please Specify):
Requested by (Contact No. & Designation and Signature): Contact Number: Email ID:

<b>Results:</b>						
<b>Microscopy</b> ( <input type="checkbox"/> ZN <input type="checkbox"/> Fluorescent)			<b>Test ID</b>			
Lab ID No	Visual appearance	Negative	Scanty	Result	1+	2+
Sample A	S M H					
Sample B	S M H					
Date tested:		Date Reported:		Reported by: (Name and Signature)		
Laboratory Name:						

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

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Appendix 13A

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

<b>Patient Information</b>		
Patient name	Age (in yrs):	Gender: <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/> OTH
Patient mobile no. or other contact no.	Specimen collection date (month):	<input type="checkbox"/> Sputum <input type="checkbox"/> Other (specify):
Aadhaar no./A-cards		
Patient address with landmark	HIV Status: <input type="checkbox"/> Negative <input type="checkbox"/> Non-Response <input type="checkbox"/> Unknown Key populations: <input type="checkbox"/> Contact of known TB Patient <input type="checkbox"/> Contact of known DR TB Patient <input type="checkbox"/> Diabetes <input type="checkbox"/> Tobacco <input type="checkbox"/> HIV/AIDS <input type="checkbox"/> Migrant <input type="checkbox"/> Refugee <input type="checkbox"/> Urban slum <input type="checkbox"/> Health-care worker <input type="checkbox"/> Other (specify):	

Name and Type of referring facility (PHAC/CTC/DTC/DC/MT/MD/MS/College/DR-TB Centre/RS/CPWS/Other, specify): Health Establishment ID (NHSAY): State: District: Tuberculosis Unit (TU):	Type of patient: <input type="checkbox"/> Public sector <input type="checkbox"/> Private sector Episode ID:
---	--

<b>Reason for Testing</b>	
<b>Diagnosis and follow up of TB</b>	
Diagnosis of TB (for presumptive TB) HIO anti TB Rx for >1 month: <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> TB asymptomatic <input type="checkbox"/> Any abnormality in X-ray <input type="checkbox"/> Repeat Exam <input type="checkbox"/> Presumptive/NTM	Follow up (Smear and culture) Reason: <input type="checkbox"/> End IP <input type="checkbox"/> End OP Post treatment: <input type="checkbox"/> 6m <input type="checkbox"/> 12m <input type="checkbox"/> 18m <input type="checkbox"/> 24m Predominant symptom: Duration: days

<b>Diagnosis and follow up Drug-resistant TB</b>	
Diagnosis of DR TB (DRT/ DST) <input type="checkbox"/> New <input type="checkbox"/> Previously treated Presumptive MDR TB <input type="checkbox"/> AI TB diagnosis <input type="checkbox"/> Follow up Sm+ve DR TB <input type="checkbox"/> Presumptive H monotherapy Presumptive XDR TB <input type="checkbox"/> MDR/RR TB at Diagnosis <input type="checkbox"/> Failure of MDR/RR TB regimen <input type="checkbox"/> Recurred case of second line treatment	Follow up (Smear & culture) Treatment follow up month: Type of case: <input type="checkbox"/> H monotherapy TB <input type="checkbox"/> MDR TB <input type="checkbox"/> XDR TB Regimen Type: <input type="checkbox"/> All oral H monotherapy TB regimen <input type="checkbox"/> Single MDR TB regimen <input type="checkbox"/> All oral longer regimen <input type="checkbox"/> Any other regimen Regimen composition: <input type="checkbox"/> Lx <input type="checkbox"/> Mx <input type="checkbox"/> Bx <input type="checkbox"/> Sx <input type="checkbox"/> Cx <input type="checkbox"/> Ds <input type="checkbox"/> E <input type="checkbox"/> F <input type="checkbox"/> Gx <input type="checkbox"/> Hx <input type="checkbox"/> Ix <input type="checkbox"/> Jx <input type="checkbox"/> Kx

Test requested: <input type="checkbox"/> Microscopy (TST) <input type="checkbox"/> DNA <input type="checkbox"/> Chest X-ray <input type="checkbox"/> Cytopathology <input type="checkbox"/> Histopathology <input type="checkbox"/> CRNAAT <input type="checkbox"/> TruNAAT <input type="checkbox"/> Culture <input type="checkbox"/> DST <input type="checkbox"/> FL-LPA <input type="checkbox"/> SL-LPA <input type="checkbox"/> Gene Sequencing <input type="checkbox"/> Other (Please Specify):
Requested by (Contact No. & Designation and Signature): Contact Number: Email ID:

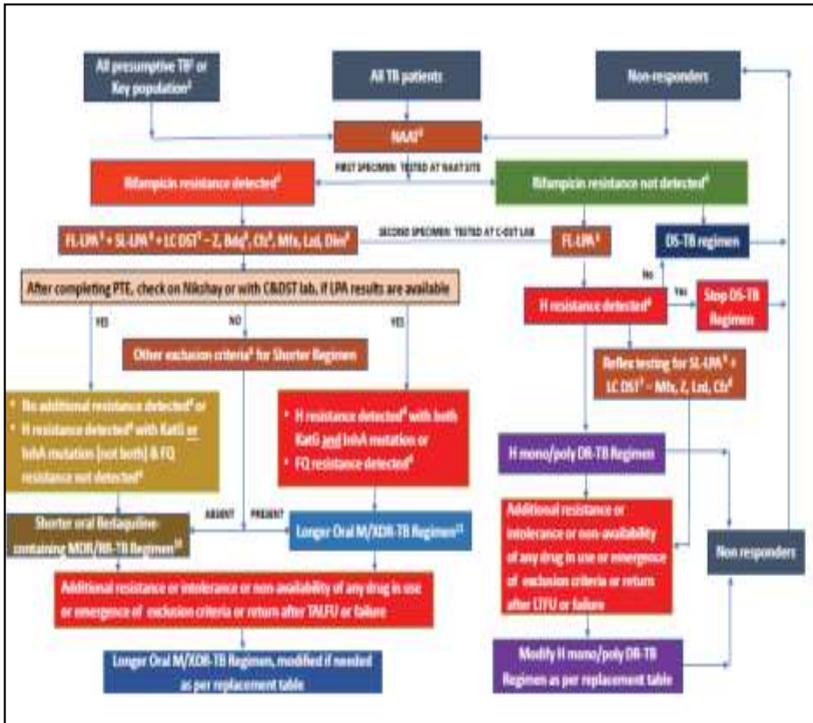
<b>Results:</b>						
<b>Microscopy (<input type="checkbox"/> ZN <input type="checkbox"/> Fluorescent)</b>				<b>Test ID</b>		
	Lab ID No	Visual appearance	Negative	Scanty	Result	
Sample A	S	M	H		1+	
Sample B	S	M	H		2+	3+
Date tested:		Date Reported:		Reported by: (Name and Signature)		
Laboratory Name:						

**APPENDIX 8 (contd)**  
**LABORATORY FORM FOR XPERT MTB/RIF ASSAY TEST**  
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**ICMR Specimen Referral Form for COVID-19 (SARS-CoV2)**

**INTRODUCTIONS**

This form is for collection centers/ 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 centers/ labs exercise caution to ensure that correct information is captured in the form.

**INSTRUCTIONS**

- Inform the local / district / state health authorities, especially surveillance officers for further guidance
- Seek guidance on requirements for the clinical specimen collection and transport from local officer
- This form may be filled in and shared with the ICMR 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

ICMR Form ID: \_\_\_\_\_

**A.2 PERSONAL DETAILS**

\*Patient Name: \_\_\_\_\_ Father's Name: \_\_\_\_\_

\*Age: \_\_\_\_\_ Years/Months/ Days (If age <1 yr, pl. tick month/ days checkbox)

\* Gender: Male  Female  Transgender

\*Occupation: Health Care Worker  Police  Sanitation  Security Guard  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 two)

\*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

\*CoMUN Biometric ID (If available, also have it Blank): \_\_\_\_\_

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

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

AZD1225 (Johnson & Johnson)  Covovax (Serum Institute of India)  Zycov-D (Zydus Cadila)

SUN-CoV273 (Novartis)  Corilo (NovPharm)  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: Nasal Swab  Blood Swab  Bronchoalveolar Lavage  Endotracheal Aspirate  Respiratory secret

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

\*Name of test 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 Test/No: CRVAAT lab)

\* 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, E-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 case/ Non-containment case/ Point of entry/ Testing on demand

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**\*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 B1)
- 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 asterisk 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>B.1 CLINICAL SYMPTOMS AND SIGNS</b>					
Cough	<input type="checkbox"/>	Loss of taste	<input type="checkbox"/>		
Sore Throat	<input type="checkbox"/>	Diarrhoea	<input type="checkbox"/>		
Fever	<input type="checkbox"/>	Breathlessness	<input type="checkbox"/>		
Loss of smell		Other symptoms, please specify:	_____		
Age of onset of First Symptom(M/mm/yy):	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
<b>B.2 PRE-EXISTING MEDICAL CONDITIONS</b>					
Diabetes	<input type="checkbox"/>	Over weight/ Obesity	<input type="checkbox"/>		
Heart disease	<input type="checkbox"/>	Hypertension	<input type="checkbox"/>		
Chronic Lung disease	<input type="checkbox"/>	Cancer	<input type="checkbox"/>		
Chronic Kidney Disease	<input type="checkbox"/>	Any other please specify:	_____		
<b>B.3 HOSPITALIZATION DETAILS</b>					
Hospitalized: Yes	<input type="checkbox"/>	No	<input type="checkbox"/>	Hospital State:	_____ Hospital
Hospitalization Date:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	District:	_____
				Hospital Name:	_____
<b>TEST RESULT (To be filled by Covid-19 testing lab facilities)</b>					
Date of sample receipt(M/mm/yy)	Sample accepted/ Rejected	Date of Testing (M/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,  
5<sup>th</sup> floor, MSB.**

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