

Laboratory Services
Dept. of Microbiology
Seth G.S Medical College and K.E.M Hospital
Primary Specimen Manual



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(Collection, Handling and Transport)

(KEM / TP 1 / PSM)

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Professor and Head

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40	HOD*, Urology	
41	HOD*, Radiology	
42	Medical Officer-in-charge, ART Centre	

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 @ kem.edu.

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List of abbreviations

Abbreviation used	Full form
Ab	Antibody
AFST	Antifungal Susceptibility Test
ART	Anti-Retroviral Therapy
ASO	Anti Streptolysin O
CBWTF	Common Biomedical Waste Treatment Facility
CMV	Cytomegalo virus
COVID	Corona Virus Disease
CRBSI	Catheter Related Blood Stream Infection
CVTS	Cardiovascular and Thoracic Surgery
Div.	Division
DOT	Directly Observed Treatment
DST	Drug Susceptibility test
Elab	Emergency Laboratory
ELISA	Enzyme Linked Immunosorbent Assay
ENT	Ear, Nose and Throat
EPTB	Extra-pulmonary Tuberculosis
GAS	Group A Streptococci
GI	Gastrointestinal
HAV	Hepatitis A virus
HEV	Hepatitis E virus
HH	HBsAg and Anti IgM to Hep C
HHH	HIV antibody, HBsAg and Anti IgM to Hep C
HOD	Head of Department
hrs	Hours
ICTC	Integrated Counselling and Testing Centre

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ILI	Influenza like illness
JE	Japanese B encephalitis
MIC	Minimum Inhibitory Concentration
MSB	Multi-storeyed Building
NTEP	National Tuberculosis Elimination Programme
OBGY	Obstetrics and Gynaecology
Ped	Paediatric
PPE	Personal Protective Equipment
PSM	Preventive and Social Medicine
PTB	Pulmonary Tuberculosis
qPCR	Quantitative PCR
RAT	Rapid Antigen Test (COVID-19)
RDT	Rapid Diagnostic Test
RDT	Rapid Diagnostic Test
RF	Rheumatoid factor
RPR	Rapid Plasma Reagin
RT-PCR	Reverse transcriptase Polymerase Chain Reaction
SRF	Specimen Referral Form
TAT	Turnaround time
TT	Tetanus toxoid
V.D.R.L	Venereal Disease Research Laboratory
VRDL	Viral Research and Diagnostic Laboratory
VZV	Varicella zoster virus
WGS	Whole Genome Sequencing

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1. **FOREWORD**

This Primary Specimen Manual has been prepared to provide an overview of the tests offered, their indications and limitations and also facilitate the process of aseptic and standardized collection and transportation of clinical specimens for microbiological investigations. This 8th issue has incorporated COVID-19 RT-PCR, RAT and COVID-19 antibody tests in addition to the HHH rapid tests in Elab. The soft copy is available online at kem.edu.

2. **INTRODUCTION**

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

The Department of Microbiology offers diagnostic services for infectious diseases through its different divisions viz. Clinical Bacteriology, Molecular Diagnostics, Mycobacteriology, Mycology, Parasitology, Serology, and Virology & Immunology including ICTC. Apart from these divisions, the department also offers emergency laboratory services after routine hours for processing specimens of emergency nature or from seriously ill patients (refer section 5a) . 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 should also have completed their COVID-19 vaccination series .

QUALITY ASSURANCE

Services are provided using approved reagents and kits, calibrated equipment and controls, and trained and competent manpower, supervised and authorized by

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qualified clinical microbiologists. External Quality Assessment and continual improvement programs are in place to assure the quality of the results generated. The laboratory is approved by ICMR for COVID-19 related RT-PCR test.

SCOPE

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

PURPOSE

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

RESPONSIBILITY

a) *Health care workers*

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

b) *Hospital administration*

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- Provide the containers and PPE as required for collection and transport
- Facilitate immunization of health care workers
- c) Head of Laboratory
 - Sensitize health care workers on procedures described in the manual through designated staff
 - Make a copy of the manual available to all the departments
- d) Microbiology Supervisory Staff and Division in charge
 - Periodically audit compliance and suitability of the procedures
 - Take corrective action in case non-compliance is detected

3. **STANDARD PRECAUTIONS (collection, handling, transport)**

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

- All clinical specimens should be considered as potentially infectious.
- All cuts and dressings should be completely covered with impervious dressing.
- Appropriate personal protective equipment should be worn while performing collection as per expected exposure risk (e.g. a pair of clean gloves).
- Hands should be washed before and after a procedure irrespective of glove use.
- Where there is a risk of splash occurring, face shield and gown should be worn in addition.
- Follow safe injection practices. Wear a surgical mask when performing lumbar punctures.
- N95 respirators should be used while collecting throat swabs / nasopharyngeal swabs from patients with infections that are transmitted by droplets such as suspected flu, diphtheria, COVID-19 etc.
- N95 respirators should be used while collecting specimens using a bronchoscope from patients with infections that are transmitted by droplet nuclei such as flu, tuberculosis, COVID-19 etc.

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- All spills of blood and body fluids should be decontaminated with an absorbent containing 0.5-1% sodium hypochlorite (freshly prepared) immediately.(Refer pg 44)
- Used items must be discarded as per KEMH waste disposal policy (refer section 9).
- 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 collecting samples as mentioned above or where there are likely to infectious aerosols s generated. Practicing appropriate donning and doffing of PPE is essential to prevent transmission to self and others.

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.
Molecular lab /VRDL	All days except Sundays and other OPD holidays	Patients’ samples (as per latest advisory) will be accepted till 4 pm on working days.

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	Serology, Clinical	During the entire working period

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/ Aspirated pus/ Tissue / Ocular specimens / E.N.T specimens, Stool for cholera	Bacteriology, Mycology, Mycobacteriology and Parasitology	
Urine, Stool (other than for cholera) and Sputum	Clinical Bacteriology	9.00 a.m. – 11.00 a.m.
Direct walk in clients	Virology and Immunology / ICTC	9.00 a.m to 4.00 p.m
Molecular diagnosis / VRDL	Molecular Diagnosis	Patient samples will be accepted from 9.00 a.m to 4.00 p.m
Specimens / tests of emergency nature as listed in 5.b	Emergency laboratory, 7th floor, MSB	Weekdays – 4.00 p.m to next day 9.00 am Saturdays and Bank holidays – 1.00 p.m to next day 9.00 a.m Sundays and other OPD holidays – 9.00 a.m to next day 9.00 a.m

5. TESTS / SERVICES OFFERED:

Division / Location	Tests offered	Specimen type * and number where applicable	Contact Person with intercom number
Clinical Bacteriology	- Microscopy & Culture for aerobic bacteria and anaerobic bacteria -Antimicrobial susceptibility test on clinically relevant aerobic bacteria -MIC by (i) Vitek2 (as per availability) or E-test strips -Rapid identification of culture isolates by (i) Vitek 2 (ii) Vitek MS Prime (MALDI ToF) -Environmental sampling and sterility	All specimens collected aseptically in sterile containers	Dr. Lona Dash 7552 Dr. Pradnya Kale Dr. Shreeraj Talwadekar Dr. Akshay Karyakarte Dr. Priyank Trivedi 7527

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	assurance tests as required		
	BACTEC Aerobic plus for adults (as per availability)	Blood x 2 bottles each with 7-8 ml of blood	
	BACTEC Peds plus for children / neonates (as per availability)	Children- Blood x 2 bottles each with 3-5 ml blood Neonates 1-2 ml	
Molecular Diagnosis Virology division	HIV viral load for patients referred from ART	Whole blood in EDTA evacuated tube	Dr. Chaya.A.Kumar 7039 Dr. Shashir Wanjare Dr. Vaishali Surase Dr. Ranjana Thate 7822/7825
	HBV viral load (for patients referred from GI OPD)		
	HCV viral load (for patients referred from GI OPD)		
Molecular Diagnosis – VRDL division	COVID-19 RT-PCR	Nasopharyngeal swab + oropharyngeal swab OR Nasal swab + Throat swab OR Sputum in case of pneumonia	Dr. Gita Nataraj Dr. Priyanka Prasad 7552
	Influenza +/- RSV PCR (as per availability)	Same as above	
	Respiratory bacterial panel PCR (as per availability)	Nasopharyngeal swab / Sputum / Endo tracheal aspirate / BAL from a patient with clinically compatible upper or lower respiratory tract infection	
	Respiratory virus panel PCR (as per availability)	Same as above	
	CMV quantitative PCR for nephrology patients and renal transplant recipients and hematology patients	Whole blood in EDTA evacuated tube	

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	CSF neurovirus panel (as per availability)	CSF in a case of clinically compatible illness with meningitis / meningoencephalitis and radiological features of CNS abnormality and altered CSF biochemistry and cellular parameters	
	CSF bacterial PCR panel (as per availability)	Same as above	
	Zika virus PCR on blood and serum	Undifferentiated febrile illness with fever / rash/ arthralgia / conjunctivitis with or without neurological features and epidemiologically linked	
	Tropical fever panel (as per availability)	Serum / plasma from patients with acute febrile illness (undifferentiated)	
	Dengue serotyping (as per availability) for surveillance	Serum / plasma from patients who have tested Dengue NS1 positive by ELISA	
	Hepatitis A virus IgM ELISA	Patients suspected of infectious hepatitis	
	Hepatitis E virus IgM ELISA	Same as above	

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	Japanese encephalitis IgM ELISA	Patients suspected of JE	
	Varicella Zoster virus IgM ELISA	Patients suspected of VZV	
	Scrub typhus IgM antibody ELISA	Undifferentiated acute febrile illness	
Molecular diagnosis – TB (5th floor, MSB)	Xpert MTB/RIF ** assay# for simultaneous detection of MTB and Rif resistance as per	Sputum specimen x 2 / GL x 2-3 / Extra pulmonary in Falcon tube (procured from DOTS centre, 5 th floor CVTS building)	Dr. Swapna Kanade Dr. Swapnil Thombre 7827
	programmatic recommendations	(Filling of NTEP requisition form and provision of falcon tube to be done at DOTS centre, 5 th floor CVTS building)	
	TrueNAT TB assay for detection of MTB and Rif resistance	Same as above	
Mycobacteriology Test requisition form and container to be collected from DOTS centre, 5 th floor, CVTS building	Microscopy (LED fluorescent microscopy / ZN stain as per availability)	Sputum** – at least 2 specimens of which one is early morning and the other is spot for diagnosis. Only one specimen for follow up patients	Dr. Swapna Kanade Dr. Swapnil Thombre 7827
	MGIT culture and DST for 1 st and 2 nd line drugs (certified by CTD)	Follow up cultures only as per program (NTEP) recommendations	
Mycology 5 th floor, MSB	Microscopy , Culture, Identification for fungi, AST for yeasts (Vitek-2 / sensititre) as per availability	All specimens collected aseptically in sterile containers	Dr. Chaya.A.Kumar

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	-Rapid identification of culture isolates by (i) Vitek 2 (ii) Vitek MS Prime (MALDI ToF)	On culture isolates only	7039 Dr. Pallavi Surase Dr. Mitali Solanki 7857 / 7832
Parasitology	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. Vasant Baradkar Dr. Alpana Wagh 7857/7824
	Trichrome stain microscopy -Stool, duodenal aspirate, sigmoidoscopy scraping for detection of intestinal protozoan parasites	- . Stool - Duodenal aspirate - Sigmoidoscopy scraping	
	RDT - malarial antigen	Whole blood / finger prick	
Serology (5th floor, MSB)	ASO	Whole blood collected in clean, dry, plain test tube / red top evacuated tubes / yellow top evacuated tubes (with clot activator jelly)	Dr. Vijaya Torane Dr. Sonal_Thavare 7984
	Chikungunya IgM antibody (ELISA)	Same as above	
	Dengue – NS1 antigen (Rapid / ELISA)	Same as above	
	Dengue – IgG and IgM antibodies (Rapid / ELISA)	Same as above	
	Leptospirosis – IgM Antibodies (Rapid / ELISA)	Same as above	
	RF	Same as above	
	RPR / V.D.R.L	Same as above	
	Widal	Same as above	

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Referral of specimens to PCR laboratory at Kasturba Hospital via serology	Leptospirosis, Dengue	Whole blood in EDTA evacuated tubes	
Virology and Immunology 5 th floor, MSB –	ICTC @ HIV – antibody detection HCV – antibody detection HBsAg detection RPR	Whole blood collected in clean, dry, plain test tube / yellow or red evacuated tube	Dr. Chaya.A.Kumar 7039 Dr. Shashir Wanjare Dr. Vaishali Surase Dr. Ranjana Thante Dr. Deepika Gunvir 7822/7825
	CD4 count enumeration	EDTA evacuated tube	

Investigations available in Emergency Microbiology Laboratory (7th floor, MSB) 7527

- Blood and body fluids for bacterial and fungal culture
- Tissue / Pus for gas gangrene
- Intra-op tissues and products of conception for culture
- HVS culture from ANC women in labour
- Stool – hanging drop and culture for *V.cholerae*
- Samples from suspected diphtheria cases – microscopy (Gram’s and Albert’s stain) and culture
- Ophthalmic samples for microscopy and culture
- CSF for India ink
- Leptospira IgM – rapid test
- Dengue NS1 and Dengue antibody – rapid test
- Malaria – rapid antigen test
- HIV, Hepatitis B, Hepatitis C (HHV) rapid test

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6. Test indications and limitations

Sr.no.	Specimen / test performed	Indications (major)	Limitations
CLINICAL BACTERIOLOGY DIVISION			
1	Blood culture (conventional) Aerobic culture & Antimicrobial susceptibility test	CRBSI, Enteric fever, Infection of prosthetic material (implants), Infective endocarditis (IE), Meningitis, Osteomyelitis, Pneumonia, PUO, Septicemia	Usually positive only in acute phase. Multiple specimens required in IE. Lesser volumes (<10-20 ml) decrease yield. Blood culture contamination during collection can lead to pseudobacteremia.
2	Blood culture (Automated method BACTEC 200 FX) Rapid aerobic bacterial culture by automated system	Same as above If patient on antimicrobial, collect just before the next dose is due.	Pre-incubation of automated blood cultures reduces the yield of Pseudomonas, Streptococcus and Candida spp. In case of delay, store at room temperature (20-30°C)
3	Normally sterile body fluids – culture C.S.F, Pleural, Pericardial, 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	Throat swab from suspected diphtheria case Smear examination by microscopy for Diphtheria Culture on appropriate media	Suspected diphtheria	Microscopy – unreliable A positive culture followed by demonstration of exotoxin production is the gold standard
5	Sputum - Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired	Both sensitivity and specificity are considered $\leq 50\%$ unless expectorated sputum is purulent.
6	Respiratory samples culture (mini BAL, BAL, endotracheal aspirate) Smear, Culture and Antimicrobial susceptibility test	Lower Respiratory tract infections, community / hospital acquired Counts $\geq 10^4$ cfu/ml correlates better with disease though not always	Difficult to distinguish colonization from infection even with quantitative cultures. Clinical correlation essential.
7	Miscellaneous (Pharyngeal swabs, Skin scraping) Smear, Culture and Antimicrobial susceptibility test	Suspected streptococcal pharyngitis, Localised skin infections	Used to rule in disease. Collect samples in suspected GAS infection patients from posterior pharyngeal wall and tonsils. The isolate needs to be clinically correlated for its significance as a colonizer / pathogen. Swabs need to be transported to lab immediately. A dried swab is detrimental to growth and can give false negative results.

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	Test	Indications	Limitations
8	Ocular specimens (conjunctival swab, Corneal scrapings, corneal button, eye discharge, vitreous humor, cornea) Smear, Culture and Antimicrobial susceptibility test	Conjunctivitis, corneal transplant, corneal ulcer , other eye infections trachoma,	Negative microscopy or culture does not rule out disease. Bedside inoculation on appropriate media improves yield provided aseptic practices are followed.
9	Pus Smear, Culture and Antimicrobial susceptibility test	Localised skin or organ specific	Sensitivity – 70% Specificity - High
10	Wound swab Smear examination by microscopy	Bacterial cellulitis, gas gangrene	Microscopy and culture unreliable. Collect tissue material or purulent discharge whenever possible.
11	Tissue (other appropriate specimen) for gas gangrene Smear and Culture (anaerobic)	Gas gangrene, local infection, intra-operative. Bedside inoculation in RCM medium is recommended. RCM to be collected from 7 th floor, Bacteriology (Media room) division	Gas gangrene is a clinical diagnosis. Microscopy cannot characterize the genus. A negative test does not rule out disease. Specimen collected without appropriate debridement will yield contamination / false negative result.
12	Specimens from female genital tract (Vaginal /cervical swab, Urethral discharge, product of conception) 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	Stool Microscopy – hanging drop	Diarrhoeas, purulent enterocolitis	A negative test for darting motility does not rule out cholera (sensitivity and specificity ~ 60%)
14	Stool Culture & Antimicrobial susceptibility test	Diarrhoeas, dysentery, purulent enterocolitis	Necessary to process specimens immediately to prevent overgrowth by normal flora.
15	Urine Smear, culture & Antimicrobial susceptibility test	Recurrent / Complicated UTI Known UTI with treatment failure PUO Asymptomatic bacteriuria in pregnant women	-False positives with clean catch urine specimens is high since the urine sample passes through the distal urethra and can become contaminated with commensal bacteria. -Culture of urine from urine collection bag gives false positive result. -Culture positive urine in a sick patient does not exclude another site of serious infection. -Prior antibiotic therapy may lead to negative urine culture in patients with UTI. -Sterile pyuria maybe due to causes other than non-fastidious aerobic bacteria.

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SEROLOGY DIVISION			
	Test	Indications (major)	Limitations
16	RF Test for rheumatoid factors	In-vitro detection of Rheumatoid factor in patients serum by latex agglutination method.	-Does not provide definite diagnosis of rheumatoid arthritis and should always be correlated clinically -False positive results are seen in auto immune diseases, acute bacterial and viral diseases - Test can be negative in some patients with Rheumatoid arthritis.
17	ASO test	Detection of antibodies to streptolysin O produced by group A beta hemolytic streptococci by latex agglutination method.	-All positive results should always be correlated clinically -Nonspecific results are seen in lipemic, hemolysed, contaminated and high protein content serum -False positive results are seen with the use of plasma instead of serum
18	RPR / VDRL Test	For detection and quantification of reagin antibody in serum/plasma and spinal fluid in syphilitic patients.	-Nonspecific test for syphilis - All positive results should be correlated clinically -All positive samples should be confirmed by TPHA or FTA ABS - False Negative: early primary syphilis; in secondary syphilis because of prozone reaction; and in some cases of late syphilis.
			-Biological false positive occurs in conditions such as -infectious mononucleosis, viral pneumonia, malaria, lepromatous leprosy, pregnancy, collagen disease, other autoimmune diseases
19	Widal Test	Detection of typhoid fever or paratyphoid fever by agglutination method.	-Not a specific (65%) or sensitive test (65%) -All reactive titres should be correlated clinically - TAB vaccinated patients may show high titres
20	Leptospira IgM rapid	Qualitative detection of IgM class of Leptospira specific antibodies in human serum/ plasma/whole blood by rapid immunochromatography method.	- Less specific than ELISA -All positive results should always be correlated clinically -Intensity of test line depends on the stage of the disease and titre of the antibody -Samples collected during early stage of disease (0-7days) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
21	Leptospira IgM ELISA	Qualitative detection of IgM class of antibodies against Leptospira by ELISA method.	Same as above

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22	Dengue NS1 – Rapid (As per notification received from MoHFW, GoI, a positive result by rapid test will be considered probable due to its poor sensitivity and specificity)	Qualitative detection of non-structural protein 1 (NS1) of dengue virus in serum/plasma by rapid immunochromatography method during first week of illness.	Samples collected during late stage of disease (after 7 - 9 days of fever) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
23	Dengue NS1 – ELISA (In a clinically compatible case, demonstration of NS1 antigen by ELISA is considered confirmatory)	Same as above	Same as above
24	Dengue IgG/IgM Rapid (As per notification received from MoHFW, GoI, a positive result by rapid test will be considered probable due to its poor sensitivity and specificity)	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 of the disease and titre of the antibody -Samples collected during early stage of disease (0-7days) may yield negative results Positive results of rapid tests to be confirmed by ELISA.
25	Dengue IgM ELISA (In a clinically compatible case, demonstration of Dengue IgM antibody by ELISA is considered confirmatory)	Same as above	Same as above
26	Chikungunya IgM ELISA (as per availability of kits) (In a clinically compatible case, demonstration of Chikungunya IgM antibody by ELISA is considered confirmatory)	Qualitative detection of IgM class of antibodies against Chikungunya virus by ELISA method.	All positive results should be correlated clinically
27	Scrub typhus IgM ELISA	Qualitative detection of IgM class of antibodies against Scrub typhus by ELISA method.	All positive results should be correlated clinically

MYCOBACTERIOLOGY DIVISION
(Also refer to Appendix 9)

Sr.no.	Specimen / test performed	Indications (major)	Limitations
27	Microscopy	Clinical suspicion of PTB / EPTB	Sensitivity low (10 ⁵ orgs/ml)
28	Culture	All EPTB cases and suspected MDRTB cases as per recent PMDT guidelines	Liquid culture - contamination
29	XpertMTB/RIF assay	Initial diagnostic tests for MDRTB suspects, pediatric TB, all HIV positive TB suspects and all extrapulmonary TB	Detects rifampicin resistance only. Cannot predict for other anti-TB drugs other than INH.

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PARASITOLOGY DIVISION			
Sr.no.	Specimen / test performed	Indications (major)	Limitations
30	Stool / other specimens – Microscopy Trichrome stain microscopy - Stool, duodenal aspirate, sigmoidoscopy scraping for detection of intestinal protozoan parasites	Suspected protozoan 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.
31	Blood – RDT malarial antigen	Clinically suspected malaria cases	- Detection limit is usually 200 parasites / μ 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> .
MYCOLOGY DIVISION			
Sr.no.	Specimen / test performed	Indications (major)	Limitations
32	Any specimen – Microscopy(KOH)	Suspected superficial or deep fungal infection	-The sensitivity of a KOH prep is relatively low (20-75%) -The test may require overnight incubation for complete disintegration of thicker specimens like hair, nail, or biopsy
33	Microscopy – India ink	Suspected cryptococcal infection	-The diagnosis of <i>C. neoformans</i> by India ink staining should be considered a presumptive result -Culture, biochemical 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
33	Culture	Suspected superficial or deep fungal infection	-Longer time required for growth of different fungi -Contamination by saprophytic fungi

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<u>VIROLOGY AND IMMUNOLOGY DIVISION</u>			
Sr.no.	Specimen / test performed	Indications (major)	Limitations
35	HIV Antibody tests (Rapid)	<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 women who directly come in labour without any antenatal check-up</p>	<p>-False Negative result : in window period & terminal stage of HIV disease</p> <p>-False positive result: autoimmune disease, multiple blood transfusion, pregnancy etc.</p>
36	HBsAg ELISA	<ul style="list-style-type: none"> ● Signs/symptoms suggestive of hepatitis ● H/o exposure 	<p>-False Negative : during incubation period</p> <p>-False positive: due to presence of other antigens or elevated levels of Rheumatoid factor</p>
37	Anti HCV ELISA	<ul style="list-style-type: none"> ● Signs/symptom suggestive of hepatitis ● H/o exposure 	<p>-False Negative: in window period</p> <p>-False positive: elevated levels of Rheumatoid factor</p> <p>- Cannot differentiate recent from past infection</p>
38	RPR test	<ul style="list-style-type: none"> ● Direct walk in patients with high risk behavior ● Patients referred by the STI counselor 	-See page 22 above
39	CD4 count	<ul style="list-style-type: none"> ● HIV positive patients referred from the ART centre 	-Nonspecific marker which can be affected by many other conditions

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Sr.no.	Specimen / test performed	Indications (major)	Limitations
MOLECULAR DIAGNOSTICS			
40	HIV viral load	Monitoring response to treatment	The detection limit (sensitivity) varies between kits. The current test has a detection limit of 150 RNA copies / ml/
41	HBV viral load	Initiate treatment and monitor response to therapy	Limit of detection 6 IU/ml
42	HCV viral load		Limit of detection 9 IU/ml
43	COVID-19 RT-PCR	Detection of nucleic acid of COVID-19 . Testing strategy as per periodic ICMR advisory. Repeat testing for confirming non-infectiousness in severely ill hospitalised COVID 19 patients.	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.
45	Influenza PCR	Detection of nucleic acid of Influenza A (ph1n1 , pn3n2), Influenza B (victoria, yamagata) in respiratory samples	The detection limit (sensitivity) varies between kits between 50 copies / reaction. 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.
46	Respiratory bacterial panel PCR	Detection of nucleic acid of <i>S.aureus</i> , <i>S.pneumoniae</i> , <i>K.pneumoniae</i> , <i>M.pneumoniae</i> , <i>Salmonella spp</i> , <i>S.pyogenes</i> , <i>Bordetella spp</i> , <i>C.pneumoniae</i> , <i>S.agalactiae</i> , <i>A.baumannii</i> , <i>P.aeruginosa</i> , <i>L.pneumophila</i> , <i>H.influenza (A-F)</i> , <i>M.catarrhalis</i> from various respiratory samples like BAL, mini-BAL, endotracheal aspirate, sputum, gastric lavage.	The detection limit (sensitivity) varies between kits. The current test has a detection limit is 10 ³ 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.
48	Respiratory virus panel PCR	Detection of nucleic acid of Parecho virus, non COVID 19 human Corona virus, Parainfluenza viruses 1-4, Influenza viruses A including subtypes, B and C, hMPV, enterovirus, Human adeno virus, RSV A/B, Rhino virus, Boca virus	The detection limit (sensitivity) varies between kits. The current test has a detection limit is 10 ³ 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.

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49	Meningitis bacterial and fungal PCR panel	<i>Hemophilus influenzae, N.meningitidis, E.coli, S.agalactiae, S.pneumoniae, L.monocytogenes, NTM, MTBC, Cryptococcus neoformans / gatti</i>	The detection limit (sensitivity) varies between kits. The current test has a detection limit <i>S.agalactiae</i> – 250 cfu / ml NTM – 150 cfu / ml MTBC 15 cfu / ml All others – 500 cfu / 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.
51	CSF Neurovirus panel	Detection of nucleic acids <i>human Adenovirus, Enterovirus, human Parecho virus, HSV1, HSV2, human Parvovirus B19, EBV, VZV, human CMV, human Herpesvirus 6 and 7</i> in CSF samples.	The detection limit (sensitivity) varies between kits. The current test has a detection limit <i>HSV 1 / 2 - 0.5 copies / µl</i> <i>VZV – 125 IU/ml</i> All others – 1.0 copy / µl 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.
	Zika virus PCR	Detection of nucleic acid of Zika virus	The detection limit (sensitivity) varies between kits. The current test has a detection limit <i>HSV 1 / 2 - 0.5 copies / µl</i> <i>VZV – 125 IU/ml</i> All others – 1.0 copy / µl 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.
52	Tropical fever panel	Diagnosis of dengue, chikungunya, malaria and salmonella spp in blood samples.	The detection limit (sensitivity) varies between kits. The current test has a detection limit <i>20 nucleic acid copies / reaction</i> 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.
53	Cytomegalovirus qPCR	Detection of nucleic acid of <i>Cytomegalovirus</i> in blood samples.	The detection limit (sensitivity) varies between kits. The current test has a detection limit (lower) <i>256 IU / ml with an upper limit of 5.0 x 10⁸ IU / ml</i> 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
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			cold chain and time to processing since receipt.
54	Hepatitis A virus IgM ELISA	Qualitative detection of IgM class of antibodies against hepatitis A virus by ELISA method	All positive results should be correlated clinically
55	Hepatitis E virus IgM ELISA	Qualitative detection of IgM class of antibodies against hepatitis E virus by ELISA method	All positive results should be correlated clinically
56	Japanese encephalitis IgM ELISA	Qualitative detection of IgM class of antibodies against JE virus by ELISA method	All positive results should be correlated clinically
57	Varicella Zoster virus IgM ELISA	Qualitative detection of IgM class of antibodies against VZV by ELISA method	All positive results should be correlated clinically
REFERRAL OF SPECIMENS			
43	Lepto PCR	Suspected leptospirosis, 1 st week, antibody negative	A negative test does not rule out disease. A positive test to be correlated clinically and with other microbiological tests. Best results when specimens tested the same day of collection. Follow triple packaging while transporting. Transport in cold chain.
44	Dengue PCR	Suspected Dengue, 1 st week, NS1 Ag and IgM Ab negative	Same as above. Does not speciate.
45	Throat / nasal swab for H1N1 influenza	Category 'C' - Patients with Influenza like illness requiring admission / admitted	Positivity is very high early in the course of disease (upto 5 days). Not recommended as a test for monitoring disease. Processing the specimen within 24 hours of collection improves yield
46	COVID-19 positive samples for WGS	State – Haffkine Institute Kasturba Hospital WGS Lab	

7. Test requisition

- Please refer to tables 4, 5 and 6 above before referring specimens for testing.

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- An appropriately / adequately filled requisition form must accompany all specimens. This should also include detailed clinical history and contact details of patient . Also mention CSF routine microscopy findings for CSF PCRs.
- The identity of the person filling the form should be legible.
- Sample requisition forms are provided as annexures in this document.
- Samples should be sent to the laboratory within two hours of collection.
- Samples for molecular diagnosis should be sent in cold chain.
- Please check the section on specimen acceptance and rejection criteria.
- The minimum details expected are
 - o sample type, site of collection as applicable (example: in case of urine whether from catheter or in case of pus , the site)
 - o date of collection, time of collection in case of blood and body fluids for culture and urine for culture
 - o name, age, gender, registration number, ward, unit, address for notifiable diseases, address and phone number for COVID-19 tests
 - o provisional clinical diagnosis
 - o any treatment that can influence the result

Urgent samples

- **Manual SRF** – while filling the SRF, samples that require to be processed urgently , should be written as ‘urgent’ on the form itself.
- **Through HMIS** – At clinical side, for samples to be processed urgently, choose the option ASAP from the priority drop down on the sample collection list page.
- Confirm that ASAP has been highlighted
- The laboratory should first check from the priority drop down menu and filter for ASAP. If such patients / samples have been identified, these should be treated as urgent, collected, accepted, processed and reported without delay as applicable.
- **Oral requests** for additional tests on a sample that is already accepted for another test, maybe considered telephonically if
 - o The sample is available

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- The sample integrity is maintained
- The test processing is valid within the acceptable time since receipt
- The quantity is sufficient for the additional test
- The person requesting should identify themselves completely

8. 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 (**Annexure 3**)
- (iv) Wear appropriate PPE.
- (v) Prepare patient as required for the collection
- (vi) Collect specimens from the actual site of infection where possible
- (vii) Collect the specimen aseptically
- (viii) Collect at the appropriate time (where recommended) and in adequate quantity
- (ix) Collect in clean, sterile, screw capped containers
- (x) Collect prior to the administration of antibiotics for culture
- (xi) Label the specimen container with date, name, registration number, ward, unit, specimen, and test required.
- (xii) Fill the requisition form – refer section 7
- (xiii) After collection, close the container and keep in upright position
- (xiv) If the outside of the container is contaminated during collection, decontaminate with 70% alcohol or 0.5% sodium hypochlorite (1:10 dilution) wipe.
- (xv) Remove PPE and discard in the red bag.
- (xvi) Wash hands and dry with a clean towel or use an alcoholic hand rub.
- (xvii) If during collection / handling / transport the specimen, the 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 (page 46).

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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.(Annexure 1 , page 49)
- Reports are issued as per the turnaround time mentioned.
- Specimens will not be stored for any other investigation.
- 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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c. Order of draw / aliquoting

Blood samples must be drawn by phlebotomists in a specific order to avoid cross-contamination of the sample by additives found in different collection tubes.

- Blood culture tube or bottle
- Sodium citrate tube (eg, blue closure)
- Serum tubes, including those with clot activator and gels (eg, red, red-speckled, gold closures)
- Heparin tube with or without gel (eg, dark green, light green, speckled green closures)
- EDTA tube with or without gel separator (eg, lavender, pearl, pink closures)
- Sodium fluoride/potassium oxalate glycolytic inhibitor (eg, gray closure)

CSF – once collected , it should be aliquoted as follows

- a. Chemistry
- b. Microbiology
- c. Cells
- d. Cytology and virology

9. DISPOSAL OF WASTE GENERATED during collection and transport

- 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 3/4th 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.

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- Regular, non-infectious waste such as packaging material should be discarded in black bags.

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

- Patients / Direct walk-in clients whose HIV status needs to be determined, go through the process of pre-test counseling, informed written consent, blood collection, testing and post-test counseling.
- HIV counselling is provided for direct walk-in-clients and OPD patients. Once informed consent is obtained, blood samples are collected for HIV testing.
- For indoor patients, an appropriately collected sample should be sent with a properly filled requisition cum consent form for HIV testing (Appendix 2, 3; pg 65, 66)
- For CD4 count enumeration, only patients referred by the ART centre are tested. Clinicians should refer HIV positive patients under their care first to ART centre who after registration at ART will be referred to Virology and Immunology Division for blood collection and testing.
- **NO SAMPLE WILL BE ACCEPTED WITHOUT A COMPLETELY FILLED REQUISITION FORM (Annexures 2-6 as appropriate)** . 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.

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- The consent form is available in English and Marathi (Annexure 2). 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, trained resident doctors, staff nurses, medical social workers, etc can carry it out. Only if the patient is willing for HIV testing, his/her blood should be collected.
- In case of minors, the consent should be obtained from the parents/guardians.
- In case of unconscious patients, where there is a need for diagnosis of HIV for management of the patient, consent should be obtained from the parents/ spouse/ closest relative available at that time.
- In case no attendant is available, the test if necessary for management may be carried out on recommendation of two attending doctors.

11. SPECIMEN COLLECTION - BLOOD – [FOR SEROLOGY, VIROLOGY , 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 collection material for evacuated tubes and tube holder, cotton ball, gloves, alcoholic hand rub solution, collection container - preferably pre-labelled [clean / sterile , dry test tube or evacuated tubes - red cap for plain blood and purple cap for EDTA], sharps can, requisition form, red bag and yellow bag.
- If multiple collections are done using the same gloves, and if the gloves are visibly clean, the same pair of gloves can be used provided the gloves are disinfected after every collection using 70% alcohol/ alcoholic hand rub.

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- In case there is contamination with blood, the gloves should be removed immediately and discarded in the red bag and replaced with new pair of plastic and latex gloves.

Procedure

- Help the patient sit comfortably on a chair with an armrest / or lie down on a bed/couch.
- Use alcoholic hand rub to disinfect your hands.
- Wear plastic and clean latex gloves. Also wear a plastic apron if required.
- Place absorbent material (cotton/gauze piece) below the patient's elbow to avoid soiling due to any leakage.
- Inform patient about the collection and the discomfort that is likely to be felt [a small prick like an insect bite].
- Pre label the collection device with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection.
- Tie a tourniquet above the site of blood collection to make the vein prominent. [This is usually above the patient's anterior cubital fossa on the forearm].
- Instruct the patient to clench his/her fist while collection is on.
- Disinfect the site of collection [patient's] with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol].
- After use, discard the alcohol swab in the yellow bag.
- Take a new sterile needle [preferably 21 G for an adult and 22 G for a child] and syringe / sterile evacuated tube set in front of the patient. The needle is attached to the syringe.
- Discard the paper/plastic cover of the syringe and needle in the blue bag.
- Insert the needle aseptically into the vein at an angle of 45 degrees.
- Allow blood to flow and collect 3-6 ml/ as per evacuated tube capacity.
- Release the tourniquet.
- Tell the patient to release the clenched fist.
- Withdraw the needle slowly and place a dry cotton swab at the puncture site.

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- 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.

12. BLOOD – FOR CULTURE [AEROBIC / FUNGAL]

- Both conventional and BACTEC blood culture bottles should be stored in the refrigerator compartment (2 -8 ° C) before use.
- Bring to room temperature prior to adding blood.
- In case of delay in transport to laboratory, store at room temperature.
- Blood collection is performed only by well-trained experienced phlebotomists (Laboratory technicians / Doctors).
- Collect blood during fever / spike phase
- Collect 7-8 ml in adults, 3-5 ml in children and 1-2 ml in neonates ensuring the required volume in each set (if available).
- 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, pre-labeled 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

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temperature if refrigerated and with the top disinfected with alcohol wipes , sharps can, requisition form, red bag and yellow bag.

Procedure

- Follow instructions as mentioned under collection of blood with the following modifications.
- Labeling - Pre label the blood culture bottle with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection.
- Site disinfection - Disinfect the site of collection with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol or chlorhexidine]. After use, discard the alcohol swab in the yellow bag.
- Follow this with disinfection with alcoholic chlorhexidine (preferred) except in children / 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. Once collected, the blood culture bottle should be stored only at room temperature in case there is a delay in transport. It should never be refrigerated.

13. 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

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- Inform the patient of the procedure
- Using aseptic precautions, collect in a 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.

14. CSF FOR CULTURE

Responsibility: Clinician

General instructions:

- The collection of CSF is an invasive technique and should be performed by experienced clinicians under aseptic conditions
- It is unsafe to do lumbar puncture in case of increased intracranial pressure
- LP should not be performed through infected skin as organisms can be introduced into the subarachnoid space (SAS)
- Clinician should explain the procedure to patient / relative if patient comatose in detail
- The container should be sterile, screw capped (available from general stores) labeled appropriately [see general instructions]. **DO NOT COLLECT IN PENICILLIN BULBS SINCE THEIR STERILITY IS NOT MAINTAINED.**
- 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

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

Procedure

- Analgesia – as recommended
- Positioning
- Position the patient at the edge of a firm bed and on one side rolled up into a ball.
- The neck is gently ante-flexed and the thighs pulled up toward the abdomen; the shoulders and pelvis should be vertically aligned without forward or backward tilt
- LP is performed at or below the L3-L4 interspace.
- An alternative to the lateral recumbent position is the seated position. The patient sits at the side of the bed, with feet supported on a chair. The patient is instructed to curl forward, trying to touch the nose to the umbilicus.
- A disadvantage of the seated position is that measurement of opening pressure may not be accurate.

Procedure

- Perform hand hygiene and wear sterile latex gloves
- Disinfect the skin with povidone-iodine or similar disinfectant and drape the area with a sterile cloth
- Inject local anaesthetic as recommended.
- Wait for 5-15 minutes
- The LP needle (typically 20- to 22-gauge) is inserted in the midline, midway between two spinous processes, and slowly advanced. The bevel of the needle should be maintained in a horizontal position, parallel to the direction of the dural fibres and with the flat portion of the bevel pointed upward; this minimizes injury to the fibres as the dura is penetrated.

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- 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 (1 1/2–2 in.) before the SAS is reached; the examiner usually recognizes entry as a sudden release of resistance, a "pop."
- If no fluid appears despite apparently correct needle placement, then the needle may be rotated 90°–180°.
- If there is still no fluid, the stylet is reinserted and the needle is advanced slightly.
- Once the SAS is reached, a manometer is attached to the needle and the opening pressure measured.
- CSF is allowed to drip into collection tubes; it should not be withdrawn with a syringe.
- Volume - 2-4 ml of CSF should be collected, the rate of collection should be slow, about 4-5 drops a second [1 ml minimum volume required for culture]
- Prior to removing the LP needle, the stylet is reinserted to avoid the possibility of entrapment of a nerve root in the dura as the needle is being withdrawn; entrapment could result in a dural CSF leak, causing headache.
- Following LP, the patient is customarily positioned in a comfortable, recumbent position for 1 h before rising,
- When the procedure is completed, the needle is removed and an adhesive bandage is placed over the injection site.
- Label the specimen as described earlier.
- Transport to the laboratory as soon as possible.

15. 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

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- Place in pre-labeled sterile container (plugged / screw capped test tube) and transport immediately.

16. EYE SWAB (CORNEAL/ CONJUNCTIVAL)

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

17. COLLECTION OF LOWER RESPIRATORY TRACT SPECIMENS

Types of specimen:

Lower Respiratory Tract Specimens include:

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

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

- a. **Sputum –expectorated**

Requirement:

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- 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 7th 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 container

- 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 (50 ml graduated screw capped sterile tubes are provided by DOTS centre, 5th floor, CVTS building)
- 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.

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

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

Sputum – Induced

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

b. Bronchial washings

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

c. Broncho alveolar lavage (BAL) culture

- The sampling area is selected based on the correspondent area of the infiltrate on chest radiograph or by the visualization of a sub segment containing purulent secretions.

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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.

18. 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

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- 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 for collection of oropharyngeal / throat swab:

- Perform hand hygiene.
- Wear appropriate mask / respirator for personal protection.
- Use a face shield.
- Wear clean / sterile gloves.
- Ask patient to open his / her mouth without putting out his tongue and to say ‘Ahhhhh...’
- While the patient is saying ‘Ahhhhh’, press down the outer two third of tongue with tongue depressor, using the left hand, enabling the tonsils and back of the throat to become visible.
- Introduce the swab with right hand between the tonsillar pillars and behind the uvula, while avoiding touching the tongue, cheeks, uvula, or lips.
- Rub the swab firmly against the inflamed part for 5 seconds while turning it round
- In case of suspected diphtheria, swab the membrane if present and If nothing abnormal is seen, swab the tonsils, the fauces and the back of the soft palate
- Take two swabs and immediately plug the same in sterile test tubes
- In case of viral aetiology, use the swabs provided with VTM. Collect samples and place the swab immediately in VTM. Close the cap and transport to laboratory in cold chain

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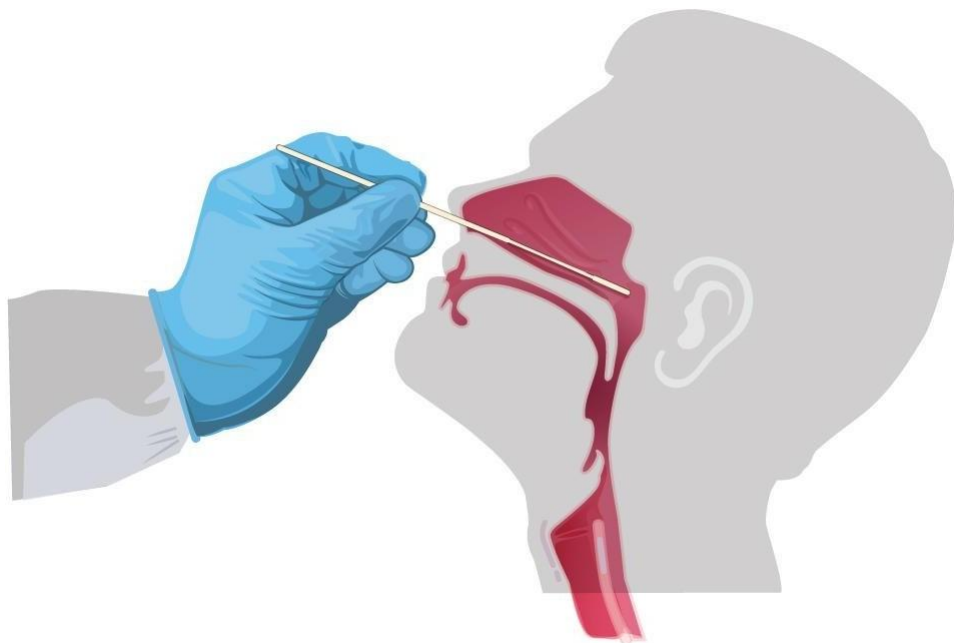
- Specimens should be transported to the laboratory immediately after labelling and properly filling up the requisition form.

Procedure for collection of nasopharyngeal swab

Requirement : PPE, Swab provided with VTM

- Remove the swab from the packaging.
- Tilt the patient's head back slightly.
- Gently insert the swab along the nasal septum, just above the floor of the nasal passage, until resistance is felt.
- The swab should reach a depth equal to the distance from the nostrils to the outer opening of the ear.
- Leave the swab in place for 5-10 seconds for secretions to be absorbed.
- Remove gently while rotating the swab.

Ref



Ref : CDC, Interim guidelines for clinical specimens for COVID-19

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19. 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.

20. PUS

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

21. 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.

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- Collect the specimen in petri dish, filter paper or clean paper.
- b) Nail
 - Clean the affected nail with spirit
 - Collect debris under the nail with scalpel in petridish
 - Pick up flakes after wetting loop with sterile saline from petridish for processing
 - If nail is avulsed then it should be cut in small pieces for processing.
- c) Hair
 - Hair should be collected from areas of scaling or alopecia
 - Clean the affected area with spirit
 - With sterilized forceps, pluck hair or stubs (at least 10-12) in grey patch or scrape with scalpel in black dot type of hair infection.
- d) Skin Biopsy
 - Decontaminate skin with 70% methylated spirit
 - Select the edge of the lesion
 - Take a biopsy with autoclaved instrument under all aseptic measures
 - Cut biopsy tissue in small pieces and crush in mortal and pestle.
- e) Mycetoma granules
 - From suspected mycetoma, look for granules in the lesions using hand lens.
 - Wash the granules in several changes of sterile distilled water
 - Crush the granules and then inoculate.
 - If granules are absent collect the purulent/necrotic material.

22. STOOL

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

23. 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

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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

24. WOUND SWAB

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

25. NEEDLE STICK INJURY PROTOCOL

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

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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 (2nd floor, main hospital building) for PEP drugs
- The medical officer at each of these places will determine risk i.e. Type of exposure and Infection Status of Source and decide on treatment
- Get Lab tests done and follow up in 3-6 months
- Follow medical officer’s advice for duration of PEP.
- It is important to initiate PEP as early as possible and within 72 hours.

26. 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.

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- Disinfect the heavy duty gloves and forceps with 1% Na hypochlorite before storage, wash well in running water and store dry.

27. SPECIMEN TRANSPORT

- All specimens should be labeled with name , reg number, ward, unit, type of specimen and date
- 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.

28. 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

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- 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.

29. SPECIMEN RECEIPT AND ACCEPTANCE

- The specimens are accepted at the reception counter for each division.
- This section is manned by a trained laboratory technician and assistant / laboratory attendant who also guides the patients for other investigations if required.
- The designated person checks transport conditions and instructs for corrections if deviations found.
- Validates 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

30. CRITERIA FOR SPECIMEN REJECTION

- Incomplete requisition
- Soiled/ blood stained requisition form (specimen is accepted; new form is asked)

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- Written consent not taken for HIV testing
- Mismatch between details on requisition form and specimen container
- No signature of clinician on requisition form
- Specimen transport time has exceeded two hours for urine culture
- Leaking or broken specimen container
- For culture, open containers
- For culture, specimen in formalin
- Specimen in wrong container
- Blood sent for culture in any other container other than blood culture bottle.
- Any sample sent for culture in penicillin bulb / yellow capped evacuated tube
- Insufficient specimen quantity (except invasive specimens)
- Hemolysed blood specimen for serology
- Lipaemic blood specimen for serology
- For culture, cotton plug contaminated with specimen
- For culture, Foley's tip.
- Dried swabs sent for culture
- Saliva instead of sputum for culture

31. REPORT DISPATCH

The reports are delivered through various modes:

- HIV reports are given to the respective direct walk-in clients/OPD patients after post-test counselling by the counsellor.
- HIV reports of ante natal clinic (ANC) patients are handed over to the counsellor working under the PPTCT (Prevention of parent to child transmission) program.
- HIV reports of indoor patients - HIV positive reports are directly handed over to the patient by ICTC counsellor after post-test counselling in the ward. All HIV negative indoor patient reports are dispatched to the referring unit.
- CD4 and HIV viral load reports are handed over to the Anti-Retroviral Therapy Centre counsellor.
- HBV and HCV viral load reports are handed over to Gastroenterology department.

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- 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 MGIT culture , DST , 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.
- **Reports generated in emergency laboratory require to be collected by the unit designated staff after the specified time.**

32. COMPLAINTS

For any complaints pertaining to any of the services offered, a note maybe sent anytime to the HOD to facilitate correction as required and improvement of services. Clinicians are also requested to fill the annual feedback forms with relevant suggestions for improvement.

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and family welfare. Government of India.

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ANNEXURE 1
Tests offered and their TAT

Division	Test Offered	Volume	TAT After specimen receipt
Virology and Immunology Division	HIV testing	3-6 ml blood in Red / yellow cap evacuated tube	24 hours
	HBsAg testing	3-6 ml blood in Red / yellow cap evacuated tube	48 hours
	HCV antibodies	3-6 ml blood in Red / yellow cap evacuated tube	48 hours
	CD4 count estimation	3-6 ml blood in a EDTA evacuated tube	24 hours
	HIV viral loads	3- 6 ml blood in EDTA evacuated tube	14 days
	Viral load – HBV & HCV	3- 6 ml blood in EDTA evacuated tube (Patient to be routed through Gastroenterology department)	14 days (reports to be collected from Gastroenterology OPD, no. 13)
Clinical Bacteriology The container for collection should be clean, sterile and screw capped or plugged and appropriately labelled.	Microscopy – Gram’s stain, Albert’s stain	1.0 ml Critical specimens – CSF, Tissue / swab for gas gangrene, Tissue / swab for Diphtheria, Pancreatic fluid, Brain abscess, Ocular specimens	1 hr
	Microscopy – Gram’s stain	Specimens other than above	4 hrs
	Hanging Drop	1 ml	30 minutes
	Aerobic culture	At least 1 ml except blood culture [refer section]	2-4 working days
	Antibiotic Sensitivity Test – aerobic bacteria Anaerobic culture		3 - 5 working days

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Division	Test Offered	Volume	TAT After specimen receipt
Clinical Bacteriology	Anaerobic culture	Sterile Swabs – soaked in exudates Tissue – NA Pus – at least 1 ml	3-5 working days
	Surveillance cultures	Exposure plates for clean rooms (such as operation theatres) and swabs from environmental and clinical contact surfaces as appropriate	24 hrs. for aerobic bacteria 72 hrs. for sporing anaerobes 5 days – 2 weeks to rule out fungal contamination

Division	Test Offered	Volume	TAT After specimen receipt
Mycology	Microscopy	Nail hair biopsy	24 hours
	Microscopy	Other	4 routine working hrs
	Culture and identification	At least 3 ml if liquid	48 hrs. – 1 month
	AFST for yeasts (as per availability)	-----	48 hrs after culture positivity
Mycobacteriology	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.

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Division	Test Offered	Volume	TAT After specimen receipt
Molecular Diagnosis / VRDL	COVID-19 RAT	Appropriately collected swabs in VTM / 2-3 ml LRT specimens in sterile containers	Next working day
	Influenza PCR	Appropriately collected swabs in VTM / 2-3 ml LRT specimens in sterile containers	Next working day
	CMV quantitative PCR for nephrology patients and renal transplant recipients (as per kit availability)	4 – 5 ml whole blood in sterile EDTA	Next working day
	Respiratory bacterial panel PCR (as per kit availability)	Appropriately collected swabs in VTM / 2-3 ml LRT specimens in sterile containers	Next working day
	Respiratory virus panel PCR (as per kit availability)	Appropriately collected swabs in VTM / 2-3 ml LRT specimens in sterile containers	Next working day
	Meningitis bacterial PCR panel (as per kit availability)	2 ml CSF in sterile container – Red evacuated tube	Next working day
	CSF Neurovirus panel (as per kit availability)	2 ml CSF in sterile container – Red evacuated tube	Next working day
	Zika virus PCR (as per kit availability)	Plasma 4-5 ml in sterile EDTA tubes (purple cap)	Next working day
	Tropical fever panel	Plasma 4-5 ml in sterile EDTA tubes (purple cap)	Next working day
	Hepatitis A virus IgM ELISA	Serum, 4 ml blood in sterile plain red / yellow cap tube	7 days
	Hepatitis E virus IgM ELISA	Serum, 4 ml blood in sterile plain red / yellow cap tube	7 days
	Japanese encephalitis IgM ELISA	Serum, 4 ml blood in sterile plain red / yellow cap tube	Next working day
	Varicella Zoster virus IgM ELISA	Serum, 4 ml blood in sterile plain red / yellow cap tube	7 days
	Scrub typhus IgM antibody ELISA	Serum, 4 ml blood in sterile plain red / yellow cap tube	7 days
Parasitology The container should be clean and screw capped.	Stool Routine & microscopy	1 tsp stool specimen	4 routine working hrs
	Malaria Antigen Detection	Whole blood in EDTA evacuated tube (3 ml)	2 hrs
	Opportunistic protozoan parasites	5 ml / 1 gm of any specimen	4 working hrs
	Microscopy- Trichrome staining	5 ml / 1 gm of any specimen	4 working hrs
Serology	VDRL/RPR		4 hrs
	RF	3-6 ml blood sample in a plain test tube/ Red / yellow capped	4 hrs

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	ASLO	evacuated tube	4 hrs
	Widal		24 hrs
	Dengue – NS1 antigen (ELISA)		72 hrs
	Dengue IgM antibody (ELISA)		72 hrs
	Leptospira IgM antibody (ELISA)		72 hrs
	Dengue – NS1 antigen (rapid)		4 hrs
	Dengue antibody rapid		4 hrs
	Leptospira IgM antibody (rapid)		4 hrs
	Chikungunya IgM antibody ELISA		72 hrs
	Dengue and Lepto PCR		6 ml blood collected in EDTA evacuated tube

Division	Test Offered	Volume	TAT After specimen receipt
Emergency Laboratory	Critical specimens / critically ill patients Microscopy Gram's stain HHH Others as per pg 18	1.0 ml	1 hr for critical specimens 2 hrs for others

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Annexure 2 SRF



Brihanmumbai Municipal Corporation
Seth G. S. Medical College & K.E.M. Hospital
Department of Microbiology
Test Requisition Form

LAB NO

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

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Annexure 3



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

Name: _____ Age: _____ Gender: M/F
Reg. No: _____ Ward No: _____ Unit: _____
Diagnosis: _____ Occupation: _____
Type of Primary Specimen: Venous Blood
Date of Specimen Collection: _____ Time of Specimen Collection: _____ am/pm
Sign of Clinician: _____

Consent for HIV Testing

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

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

Signature of Client / Parent _____ Date: _____

Counseled by (Name and signature) _____ Date: _____

एच.आय.व्ही. चाचणीसाठी लिखित संमती

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

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

आशिलाची/ पालकाची स्वाक्षरी _____ दिनांक- _____

समुपदेशकाचे नाव व स्वाक्षरी _____ दिनांक- _____

FOR LABORATORY USE ONLY

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

Lab No: _____ Sign: _____

Note:

P.T.O

NS4493HC-BMPP-52799-2021-22-1,00,000 Loose (F/B) (M-2)

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

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

टीपः

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

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Annexure 5

Annexure 15A

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

Patient Information			
Patient name		Age (in yrs): _____	Gender: <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/> TG
Patient mobile no.		Specimen collection date (DD/MM/YY) _____	<input type="checkbox"/> Sputum <input type="checkbox"/> Other (specify) _____
Other contact no.			
Aadhaar no. (if available)		HIV Status: <input type="checkbox"/> Reactive <input type="checkbox"/> Non-Reactive <input type="checkbox"/> Unknown	
Patient address with landmark		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"/> Prison <input type="checkbox"/> Miner <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 (PHI/DMC/TU/CTC/ICTC/ART/Medical College/DR-TB Centre/RBSK/Private Others, specify): _____	Type of patient: <input type="checkbox"/> Public sector <input type="checkbox"/> Private sector
Health Establishment ID (NIKSHAY): _____	Episode ID: _____
State: _____ District: _____	Tuberculosis Unit (TU): _____

Reason for Testing

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

Diagnosis and follow up Drug-resistant TB			
Diagnosis of DR TB (DRT/ DST)		Follow up (Smear & culture)	
Presumptive MDR TB	<input type="checkbox"/> New <input type="checkbox"/> Previously treated	Treatment follow up month: _____	
	<input type="checkbox"/> At TB diagnosis	Type of case: <input type="checkbox"/> H mono/poly TB <input type="checkbox"/> MDR/RR TB <input type="checkbox"/> XDR TB	
	<input type="checkbox"/> Follow up Sm+ve DS TB	Regimen Type: <input type="checkbox"/> All oral H mono/poly TB regimen <input type="checkbox"/> Shorter MDR TB regimen <input type="checkbox"/> All oral longer regimen <input type="checkbox"/> Any other regimen _____	
	<input type="checkbox"/> Presumptive H mono/poly	Regimen composition: <input type="checkbox"/> Lfx <input type="checkbox"/> Mfx ^h <input type="checkbox"/> Bdq <input type="checkbox"/> Lzd <input type="checkbox"/> Cfz <input type="checkbox"/> Cs <input type="checkbox"/> Z <input type="checkbox"/> E <input type="checkbox"/> Eto <input type="checkbox"/> Dlm <input type="checkbox"/> Am <input type="checkbox"/> Km <input type="checkbox"/> Cm <input type="checkbox"/>	
Presumptive XDR TB	<input type="checkbox"/> MDR/RR TB at Diagnosis		
	<input type="checkbox"/> Failure of MDR/RR TB regimen		
	<input type="checkbox"/> Recurrent case of second line treatment		

Test requested:

<input type="checkbox"/> Microscopy <input type="checkbox"/> TST <input type="checkbox"/> IGRA <input type="checkbox"/> Chest X-ray <input type="checkbox"/> Cytopathology <input type="checkbox"/> Histopathology <input type="checkbox"/> CBNAAT <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: _____

Results:

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

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Annexure 5

Date of specimen received: _____

Nucleic Acid Amplification Test (NAAT)		Lab serial _____	Test ID: _____
Type of test	<input type="checkbox"/> CBNAAT	<input type="checkbox"/> TrueNat	
Sample	<input type="checkbox"/> A	<input type="checkbox"/> B	
M. Tuberculosis	<input type="checkbox"/> Detected	<input type="checkbox"/> Not Detected	<input type="checkbox"/> N/A
Rif Resistance	<input type="checkbox"/> Detected	<input type="checkbox"/> Not Detected	<input type="checkbox"/> Indeterminate <input type="checkbox"/> N/A
Test	<input type="checkbox"/> No Result	<input type="checkbox"/> Invalid	<input type="checkbox"/> Error – Error Code _____ (Please arrange for fresh sample)
Date tested: _____	Date Reported: _____	Reported by: _____	
Laboratory Name: _____		(Name and Signature)	

Culture (<input type="checkbox"/> LJ <input type="checkbox"/> LC)		Lab serial _____	Test ID: _____
Lab Sr. No	Negative	Positive	NTM (write species)
			Contamination
Date Result: _____	Date Reported: _____	Reported by: _____	
Laboratory Name: _____		(Name and Signature)	

First line LPA		Lab serial _____	Test ID: _____
<input type="checkbox"/> Direct <input type="checkbox"/> Indirect		<input type="checkbox"/> Valid <input type="checkbox"/> Invalid	<input type="checkbox"/> MTB detected <input type="checkbox"/> MTB not detected
Drug	Resistant detected	Final interpretation	Remark
Rifampicin (R)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, R should not be given	
Isoniazid (Kat G)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, H(h) should not be given	
Isoniazid (Inh A)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, H(h) can be considered & Eto should not be given	
Date Result: _____	Date Reported: _____	Reported by: _____	
Laboratory Name: _____		(Name and Signature)	

Second line LPA		Lab serial _____	Test ID: _____
<input type="checkbox"/> Direct <input type="checkbox"/> Indirect		<input type="checkbox"/> Valid <input type="checkbox"/> Invalid	<input type="checkbox"/> MTB detected <input type="checkbox"/> MTB not detected
Drug	Resistant detected	Final interpretation	Remark
Levofloxacin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, Lfx should not be given. Mfx (h) can be considered.	
Moxifloxacin (h)	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes, Lfx & Mfx (h) should not be given	
Amikacin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, Am should not be given	
Kanamycin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, Km should not be given	
Capreomycin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No	If yes or inferred, Cm should not be given	
Date Result: _____	Date Reported: _____	Reported by: _____	
Laboratory Name: _____		(Name and Signature)	

Drug Susceptibility Test (DST) results		Lab serial _____	Test ID: _____																		
Lab Sr.No	1 st line drugs				SLI				FQ			Other									
	R	H (0.1)	H (0.4)	Z	E	S	Km	Cm	Am	Lfx	Mfx (0.5)	Mfx (1.0)	Mfx (2.0)	PAS	Lzd	Ctz	Bdq	Dim	Eto	Cs	Clr
Date Result: _____	Date Reported: _____	Reported by: _____																			
Laboratory Name: _____																					
R: Resistant; S: Susceptible; C: Contaminated; -- Not done																					

Other tests for TB diagnosis		Lab serial _____	Test ID: _____
Test (Please Specify): _____			
Result: _____			
Date reported: _____		Reported by: _____	
Laboratory Name: _____		(Name and Signature)	

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Annexure 6

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

***A.3.1 For Community**

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

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

A.3.2 For Hospital

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

*Fields marked with 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.1 CLINICAL SYMPTOMS AND SIGNS			
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	<input type="checkbox"/>	Other symptoms, please specify:	_____
Date of onset of First Symptom(dd/mm/yy):	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
B.2 PRE-EXISTING MEDICAL CONDITIONS			
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.3 HOSPITALIZATION DETAILS			
Hospitalized: Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
		Hospital State:	_____ Hospital
		District:	_____
Hospitalization Date:	<input type="text"/> <input type="text"/> <input type="text"/>	Hospital Name:	_____

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

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

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Annexure 7: Test requisition form for CSF PCR

Lab.no.	
Date of sample collection	
Name (patient's)	
Registration number	
Age and Gender	
Ward and unit	
Diagnosis	
Occupation	
Area of residence	
Contact number	
Date of admission	
Significant family history	
Significant disease history (self)	
History of exposure to animals	
Date of symptom onset	
Symptoms (Tick all that apply)	
Fever	Rash
Vomiting	Nausea
Abdominal pain	Diarrhoea
Cough (dry / with expectoration)	Mental confusion
Breathlessness	Seizures

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Edema	Bleeding from any site
Headache	Orbital pain
Joint pain	Sensitivity to bright light
Double vision	
CSF routine , microscopy findings	
Imaging findings	

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Clinician's signature

For any suggestions regarding this manual, please communicate to chayakumar@kem.edu
Since this is a soft copy, signatures are not appended

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