Rational Use of the Microbiology Laboratory

A Guide for Clinicians

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Preface

The Sri Lanka College of Microbiologists has addressed a long-felt need of all medical doctors by developing a book on “Rational Use of the Microbiology Laboratory - A Guide for clinicians”.

Microbiologists support patient management by ensuring quality assured pretesting, testing and post testing processes of laboratory investigations.

Although we tend to give a higher attention to testing and post-testing processes, the most important pre-test process is often neglected. Sub-standard pre-testing processes are a major setback for issuing a quality-assured report with accurate interpretation.

The preanalytical phase occurs from, as early as test selection, to the point where the sample is ready for analysis and some of the common pre-testing errors include inappropriate selection of microbiological diagnostic tests, inadequately filled request forms, errors in specimen collection, including unacceptable containers and improper handling, storage and transport conditions.

When it comes to the costs of inappropriate testing, the cost that can be attributed to the incorrect decisions made, based on test results is added to the direct cost of the unnecessary test. Hence ordering the right tests, for the right patient, at the right time not only enhances patient outcomes and safety, but undoubtedly holds economic benefits.

Hence considering the importance of the pre testing process our members have developed this guide which is based on a syndromic approach for easy reference. It outlines the microbiological investigation options available in Sri Lanka for different infectious diseases. It also provides recommendations on-selecting appropriate tests and specimen containers. The guide also covers sample collection methods, types of request forms, and the storage and transport conditions for various tests.

This will enable clinicians to accurately identify aetiological agents and initiate specific therapies, thereby improving patient outcomes and safety.

I believe this will be a practice-changing guide that will take the quality of the microbiological investigation reports to a much higher level.

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President, Sri Lanka College of Microbiologists

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Abbreviations

ABST	Antibiotic susceptibility testing
AFB	Acid fast bacilli
AMC	Anti Malaria Campaign
AOC	Amoebae, Ova, Cyst
BAL	Bronchoalveolar lavage
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
CSF	Cerebrospinal fluid
CVC	Central venous catheter
EBV	Epstein Bar virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ET	Endotracheal
EVD	External ventricular drainage
FESS	Functional Endoscopic Sinus Surgery
GC	Gonococcus
GDH	Glutamate dehydrogenase
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HVS	High vaginal swab
ICT	Immunochromatography test
IE	Infective Endocarditis
IGRA	Interferon Gamma Release Assay
MAT	Microscopic agglutination test
MAC	Mycobacterium Avium complex
MIC	Minimum Inhibitory Concentration
NP	Nasopharyngeal
MRI	Medical Research Institute
MTB	Mycobacterium tuberculosis
NAAT	Nucleic acid amplification tests
NRL	National Reference Laboratory
NSACP	National STD/AIDS Control Programme
OI	Opportunistic infections
OM	Otitis media
PCN	Percutaneous nephrostomy
PCR	Polymerase chain reaction
PJ	*Pneumocystis jirovecii*
PUO	Pyrexia of Unknown Origin
RDT	Rapid diagnostic test
RT	Room temperature
SFR	Stool full report
SJGH	Sri Jayewardenepura General Hospital
SSPE	Subacute sclerosing panencephalitis
STI	Sexually transmitted Infections
TB	Tuberculosis
TM	Tympanic membrane
TPPA	Treponema pallidum particle agglutination
VDRL	Venereal disease research laboratory
VTM	Viral transport medium

Introduction

The microbiology laboratory plays an important role in providing presumptive or confirmatory diagnosis of infectious diseases, suggesting appropriate antibiotics and monitoring response to treatment. Furthermore, it plays an active role in prevention of nosocomial infections in hospitals.

Errors in the pre-analytical phase, as in optimal timing, suitability of the test requested, specimen labelling, specimen collection and transport conditions, will result in inaccurate test results. Hence, minimizing errors in the pre-analytical phase is important to generate a reliable microbiology report.

Diagnostic stewardship involves all phases of the testing process. Effective quality improvement will help to optimize resource utilization and ensure the quality of the specimens to improve diagnosis and treatment of infections. This guideline will provide guidance to the rational use of microbiological diagnostics, enabling effective and optimal use of resources andhelping to generate a reliable microbiological report to the clinician to ensure the highest standards of patient care in the country.

Optimal time of collection, suitability of the test request, volume of the sample, type of sample and appropriate transport of sample are important for the best yield of the specimen in the laboratory. It is important to collect the most appropriate sample whenever feasible, such as pus or wound tissue rather than wound swabs, and request molecular studies and culture specimens in early illness. When collecting samples and transport in suspected infectious diseases, adhering to safety measures are of utmost important as well.

The quality of the sample does matter for a reliable test result. Proper skin cleaning prior to blood cultures, expectorated sputum instead of salivary samples, collection of midstream urine and other such measures help prevent commensals being reported as pathogens. The samples should be collected into specific containers to provide the required environment, such as sterile screw capped containers and adding sterile saline to prevent drying of samples.

A completed request form, with a brief clinical presentation, suspected clinical diagnosis and relevant exposures or contact for a particular condition and antimicrobials used are vital information to the laboratory to decide the testing method, appropriate incubation conditions, requirement for further testing and for the interpretation of test result especially in special and reference microbiology tests.

Even if you collected the samples properly, but failed to provide necessary information like site and type of the specimen, or collected insufficient volume, used improper storage and transport conditions or the label mismatched, it would result in an unsatisfactory or misleading laboratory report. Hence, providing relevant patient information and adhering to specimen storage and transport instructions are pivotal in generating an accurate laboratory report.

A separate chapter was allocated on sample collection and transport to be followed due to its influence in the test result. Liaison with the microbiology team is essential when obtaining specimens for special and reference investigations and for unrepeatable or precious samples such as CSF, bone marrow etc. The microbiology laboratory will guide you to obtain the best specimen, the transport conditions and availability of the test for the infectious disease.

Multidisciplinary team work is important when deciding clinical significance of a test result, especially to correlate the clinical history with laboratory findings before concluding on significance of the test result in some situations, such as isolation of *Candida* spp. in urine culture of a catheterized patient.

Communication with reference laboratories is of utmost important before requesting special or reference tests for infectious diseases, to know the suitability of the requested test, availability of new test methods or tests for emerging pathogens, and for optimal turn-around time of reporting. Providing an email address, fax or telephone number of a responsible person will help prevent delays in report delivery from the National Reference Laboratories.

The rational use of the microbiology laboratory, with a strong emphasis on the pre-analytical phase and diagnostic stewardship is fundamental in achieving accurate, high-quality diagnostic reports. This approach not only enhances patient care but also contributes to the broader goals of antimicrobial stewardship and healthcare cost containment.

This book provides important information for the clinicians on utilizing the microbiology laboratory rationally to ensure diagnostic stewardship and antimicrobial stewardship for high quality patient care.

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CHAPTER 1

Acute Febrile Illness

Note:

- Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Undifferentiated fever with suspected bacteremia	Blood culture Sputum/ urine/ wound swab/ pus or any other relevant specimen for - Bacterial culture	Collect specimens after obtaining detailed clinical information and physical examination and considering potential causes
Dengue infection	Blood/serum for - Dengue NS1 Antigen	Collect specimens for NS1 antigen in the first five days of fever (preferably on day 1-3) Indicate days of fever in the request form
	Rapid dengue IgM and IgG antibody test by ICT	Blood should be collected after day 5 of fever
	Rapid dengue antigen + antibody combined test by ICT	Blood should be collected day one onwards of fever, if duration of fever is uncertain
	Dengue IgM antibody test by ELISA	Blood should be collected after day 5 fever
	Dengue IgG antibody test by ELISA	Blood should be collected after day 7 of fever
	Blood/ CSF for dengue PCR	Dengue PCR is mainly performed for serotyping Specimens for molecular and antigen tests, store and transport at +4 to 8ºC
Leptospirosis	Blood/ serum/ urine/ CSF for - *Leptospira* PCR	Preferably samples should be collected within the first week of illness Transport at +2 to 8°C in a cool box
	Blood/ serum for - Microscopic Agglutination Test (MAT)	Paired specimens should be obtained in acute (day 5 onwards) and convalescent sample (10-14 days after the 1st specimen) Refer Chapter 19 - Leptospirosis

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Blood/serum for - *Leptospira* IgM detection by ELISA *Leptospira* rapid antibody test by ICT	Blood samples should be collected on day 3-5 of the illness for serology ELISA and ICT are not confirmatory tests
With respiratory symptoms ***Influenza,* COVID-19 Infection** **Other respiratory viruses**	Nasopharyngeal (NP) aspirates/ NP swabs/ throat swabs in viral transport medium for - Rapid antigen test for *Influenza* A/B and COVID-19 PCR for *Influenza* A/B and COVID-19 PCR for other respiratory viruses and atypical bacteria	Nasopharyngeal aspirates and NP swabs are more suitable specimens than throat swabs Due to sub-optimal performance characteristics of the antigen test, it is recommended to correlate test results with clinical and other relevant laboratory findings for patient management Specimens for PCR and antigen tests, store and transport at +4 to 8°C in a cool box
Enteric fever	Blood culture Stool culture Urine/ duodenal aspirate/ skin biopsy of Rosespots for - Bacterial culture Blood/ serum for - Slide agglutination test for enteric fever	Blood culture is preferable in early illness Stool culture should be performed from the second week of illness Assay is of limited clinical utility in endemic areas. Performed in few laboratories

Clinical syndrome	Microbiology investigations	Special instructions
Melioidosis	Blood culture Pus/ respiratory specimens/ radiologically-guided aspirates from abscesses for - Bacterial culture Blood/ serum for - Melioidosis antibody detection	Mention the clinical diagnosis, if melioidosis is suspected in the request form Paired samples could be obtained at any timeof the illness. Collect the 2nd sample 1-2 weeks later Test performed at the Department of Microbiology, Faculty of Medicine, University of Colombo
Rickettsial infection	Blood/ serum for - Weil-Felix antibody test Blood for - Rickettsia immunofluorescence assay Biopsy from skin lesions (eschar)/ blood in early disease for - Rickettsial PCR	This test has low sensitivity and specificity. Test performed at Bacteriology II, MRI Send 3mL blood in a sterile plain tube. Check availability before sample collection Test performed at the Department of Parasitology, Faculty of Medicine, University of Kelaniya For PCR, EDTA blood should be sent during the first week of illness to the Bacteriology II, MRI Check availability before sample collection
Malaria	Blood 2-3mL in EDTA tube or finger prick blood for - Thick and thin blood smear for microscopy Blood in EDTA tube for - Malaria antigen detection by ICT (Rapid diagnostic test (RDT)) Blood in EDTA tube for - Malaria PCR - if venous blood cannot be collected, place three blood spots (125µL each) on filter paper	Blood should be collected on suspicion, ideally during fever spikes If strongly suspected but initial test negative, do at least three consecutive blood smears and RDTs before exclusion For PCR and antigen tests, samples should be stored in refrigerator and transported in cool temperature +2 to 8°C PCR is performed in special laboratories in the Anti Malaria Campaign and University Parasitology laboratories All suspected patients must be informed to the Anti Malaria Campaign with a blood sample for reconfirmation

CHAPTER 2

Congenital and Neonatal Infections

Note:

- Request form should be duly filled with necessary details such as clinical manifestations, congenital anomalies of the neonate, relevant vaccination status and infections of the mother
- Refer to Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigation	Special instructions
Congenital cytomegalovirus (CMV) infection/disease	Blood/ serum for - CMV IgM antibody test from neonate and the mother Blood/ urine/ saliva for - CMV quantitative PCR from neonate	Collect 1mL blood from neonate within the first 3 weeks of age and 2mL blood from the mother into a sterile, plain plastic tube For PCR within the first 3 weeks - blood: collect 1mL blood into a sterile plastic EDTA tube urine: 3-5mL urine into a sterile screw capped plastic container saliva: salivary samples should be sent in a container with VTM
Congenital Rubella Syndrome	Blood for - Rubella IgM antibody from neonate and the mother Nasopharyngeal aspirate (NPA)/ NP swab/ blood/ CSF/ urine/ cataract tissue from the neonate for - Rubella PCR	Collect 1mL blood from neonate within the first 3 weeks of age and 2mL blood from the mother into a sterile plain plastic tube Send NPA in VTM container and 1mL blood in EDTA plastic sterile tube Collect CSF (0.5mL) and urine (3-5mL) into sterile screw capped plastic containers Nasopharyngeal aspirate and NP swabs are the preferred specimens for PCR Laboratory evaluation should be performed before the child reaches one year of life Contact virologist before sample collection

Clinical syndrome	**Microbiologyinvestigation**	**Special instructions**
Congenital & neonatal herpes simplex virus (HSV) disease	Blood for - HSV 1/ 2 IgM antibody test from baby and the mother If mucocutaneous lesions are present, CSF/ blood/ swabs from nose, throat, axillae, eyes, rectum in VTM for - HSV 1/2 PCR	Collect 1mL blood from baby within the first 6 weeks of age and 2mL blood from mother into sterile, plain plastic tube Collect 0.5mL CSF into a sterile plastic tube and 1mL blood into a sterile plastic EDTA tube Use Dacron or rayon swabs to collect specimens for PCR Should mention maternal HSVdisease, congenital anomalies of baby etc. in the request form
Congenital and neonatal varicella zoster	Blood for - Varicella IgM antibody test from baby and the mother Blood/ CSF/ vesicular swabs and scrapings/ scabs from crusted lesions in VTM for - Varicella PCR	Collect 1mL blood from the baby within the first 6 weeks of age and 2ml blood from the mother into a sterile plain plastic tube If antivirals are started, indicate in the request form Contact virologist for further information
Congenital parvovirus B19 infection	Blood for - Parvovirus B19 IgM antibody test from baby and the mother EDTA blood in a plastic tube for - Parvovirus PCR from the baby	Collect 1mL blood from the baby within the first 6 weeks of age and 2mL blood from the mother into a sterile plain plastic tube Mention the presence of rash, anemia in baby etc. in the request form Check availability before obtaining samples Amniotic fluid can be sent for antenatal diagnosis by PCR
Congenital Zika Syndrome Suspect if travelled to an endemic country or significant exposure to a suspected or confirmed Zika patient	Blood for - Zika IgM antibody from baby EDTA blood / CSF/ placental tissue in VTM/ urine for - Zika PCR	Samples should be sent in triple package to the virology reference laboratory, MRI Discuss with the virologist on the selection of suitable sample types and optimal time for sample collection Request form should include congenital anomalies of baby, laboratory evidence for Zika virus infection in mother, travel to high-risk countries during pregnancy etc.

Clinical syndrome	Microbiology investigations	Special instructions
Suspected Congenital syphilis	Blood/ serum for - VDRL	Send blood samples from both baby and the mother concurrently for serological assays Collect 1mL blood from the baby (not cord blood) and 2mL from the mother into a sterile plain plastic tube
	Nasal discharge/ exudate from skin lesion for - Darkfield microscopy	Collect serous exudate of skin lesions by pressing a clean cover slip on the lesion. Keep the cover slip on a clean slide. Transport the slide immediately to the laboratory at RT Refer both mother and baby to a venereologist for follow up and to confirm the diagnosis
	Antenatal screening of the mother Blood/ serum for VDRL	Testing should be at the booking visit to antenatal clinic or at least within 12 weeks of pregnancy. If not tested during pregnancy, test should be offered at the admission for delivery. Review results.
Neonatal sepsis (Bacterial)	Blood culture (in pediatric bottle)	Collect at least two peripheral venous blood (1mL) samples from two different sites and transport at RT In late onset sepsis, if there is a central line, collect blood from the central line and peripheral venous blood simultaneously For suspected necrotizing enterocolitis, do both aerobic and anaerobic blood cultures if facilities are available
	CSF for - Bacterial culture CSF bacterial antigen detection test CSF/ blood for - Bacterial Multiplex PCR	Collect CSF 0.5mL into a sterile screw capped bottle. Transport at RT for culture Sensitivity and specificity are lower in antigen tests than PCR Test is performed at the NRL Clinical Microbiology, MRI For PCR and antigen tests, collect CSF and blood into a sterile screw capped bottle and transport at +2 to 8^{o}C in a cool box

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Obtain other relevant specimens according to the organ/ system involvement as follows:	Specimens should be stored and transported to the laboratory at RT as soon as possible, best within 2 hours
	Discharge from ear, eye and umbilicus/ ET secretions/ urine/ pus/ sterile fluid aspirates for - Bacterial culture	Use separate swabs for each eye Clean catch or aseptically aspirated suprapubic urine is preferred
Suspected neonatal *Candida* infection	Blood for fungal culture	*Candida* species can grow satisfactorily in automated blood culture bottles
	Urine for - Direct microscopy for fungi Fungal culture	Clean catch or aseptically aspirated suprapubic urine samples preferred
Suspected congenital toxoplasmosis in the neonate	Blood/ serum for - *Toxoplasma gondii* specific IgM and IgG antibody test from infant and the mother	Do not collect cord blood Collect the first blood sample after day 5of life of the neonate. Paired samples should be sent 2-4 weeks apart. Transport specimens at +2 to 8ºC in a cool box If a diagnosis could not be established within the first year of life, and IgG antibodies persist beyond one year of age, it confirms congenital infection
	Blood in EDTA for - *T. gondii* specific DNA PCR	PCR is performed when the diagnosis is not established by serology Collect 2mL peripheral blood from the infant. Store and transport at +2 to 8ºC in a cool box Contact microbiologist/ parasitologist for test availability
	CSF for - *T. gondii* specific IgM/ IgG antibody test by ELISA	Collect CSF after day 5 of life of the neonate. Store and transport at +2 to 8ºC in cool box Test available at the Department of Parasitology, MRI
	CSF for - *T. gondii* specific DNA PCR	PCR is not routinely offered as a diagnostic test. Available only in few laboratories. Contact parasitologist/ microbiologist

CHAPTER 3

Diarrhoea

Note:

- Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Acute diarrhoea	Stool full report (SFR)	Usually self-limiting. A specific microbiological diagnosis is not required unless in an outbreak situation and severe infection
watery/ blood and mucous diarrhoea	Stool culture (for *Salmonella, Shigella*, *Campylobacter,* diarrhoeagenic *E.coli*)	Stool cultures are indicated in patients with fever, bloody diarrhoea, presence of red cells in the SFR, severe diarrhoea with abdominal cramps, in neonates and immunocompromised hosts Specimens are collected into clean, wide mouthed, disinfectant-free, screw capped leak-proof container with tight-fitting lid. Rectal swab should be visibly soiled with faeces
	Stool/ vomitus/ rectal swab for bacterial culture	These samples are obtained in an outbreak For the detection of *Campylobacter*, diarrhoeagenic *E. coli* or in food-borne outbreaks, specimens should be sent to the Enteric reference laboratory, MRI
In special situations	Blood culture	Obtain blood cultures in special conditions such as in infants less than 3 months with acute diarrhoea or any age with features of sepsis
Suspected viral gastroenteritis	Fresh, stool specimen for - Rotavirus antigen detection test PCR for diarrhoeagenic viruses (rotavirus/ adenovirus/ sapovirus/ astrovirus/ norovirus etc.)	Viral studies are considered in patients hospitalized with severe disease and in outbreak situations For viral studies, transport stools at +4 to 8^{o}C in a cool box
Suspected parasitological aetiology	Stool microscopy for AOC (amoebae, ova, cyst) Stool microscopy for - parasites (*Entamoeba, Giardia, Cryptosporidium*)	Contact microbiologist/ parasitologist if parasitological etiology is suspected

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Suspected Cholera	Diarrhoeal stools for direct microscopy wet mount Culture for *Vibrio cholerae*	In highly suspected patients, send a fresh diarrhoeal stool specimen immediately to the laboratory for direct microscopy For culture, send in special transport medium to the Enteric reference laboratory, MRI Transport medium is available at MRI Contact microbiologist for further information
Antibiotic-associated diarrhoea	Diarrhoeal stools for - GDH (Glutamate dehydrogenase) antigen assay *Clostridium difficile* toxin A/B assay Nucleic Acid Amplification Test (NAAT) for toxin detection	Send stools in sterile container and transport at +2 to 8^{o}C in a cool box NAAT is performed at the Bacteriology II laboratory, MRI Contact microbiologist and check the availability of tests before specimen collection
Diarrhoea in immunocompromised host		Refer Chapter 5

CHAPTER 4
Genital Infection

Note:

- Refer patients with suspected STIs to the nearest STD clinic or to a venereologist
- For further information, refer 'Sexually Transmitted Infections management guideline' published by the NSACP and Sri Lanka College of Sexual Health and HIV medicine

Clinical syndrome	Microbiology investigations	Special instructions
Urethral discharge (male)	Urethral discharge/ urethral swab for - Microscopy Gram stain (prepare the slide at bedside) Gonococcal (GC) culture (inoculate the specimen in the special media plate at bedside) Urethral discharge/ urine for - *Chlamydia*/ Gonococcal PCR	Specimens should be obtained by trained staff / venereologist Rectal/ oropharyngeal swabs may be collected depending on the clinical manifestations and sexual practices In suspected gonococcal infections, special media plates should be obtained from the nearest STD clinic/ NSACP or contact microbiology laboratory If GC culture facilities are not available, send urethral, cervical and vaginal swabs in Amies transport medium at RT to reach the nearest STD laboratory within 24-48 hours
Cervical discharge (Female)	Endocervical swab for - Microscopy Gram stain Gonococcal culture *Chlamydia*/ Gonococcal PCR	
Vaginal Discharge (for suspected candidiasis, bacterial vaginosis, PID, endometritis)	High vaginal swab (HVS) for - Microscopy Gram stain HVS culture Pus for bacterial culture	Two swabs should be collected for vaginal candidiasis, bacterial vaginosis, endometritis or PID
Suspected Trichomoniasis	Female - vaginal secretions from posterior fornix Male - urethral discharge/ centrifuged sediment of urine for - Wet mount for *Trichomonas vaginalis* (TV) Giemsa-stained smear	Contact microbiologist before specimen collection Vaginal secretions and urethral discharge should be mixed with 0.5mL normal saline in a tube for microscopy Send the specimens immediately, since it needs to be examined without delay

Clinical syndrome	Microbiology investigations	Special instructions
Epididymo-orchitis	First-pass urine / urethral swab for - Microscopy Gram stain	Refer to a venereologist if positive for GC
	Urethral discharge/ swab for Gonococcal culture	If GC is suspected, obtain slides and plates from the microbiology laboratory. If culture facilities are not available, send swabs in Amies transport medium at RT to reach the testing STD laboratory within 24-48 hours
	Midstream urine for culture	
	Urine/ urethral discharge for - *Chlamydia*/ Gonococcal PCR	
		If TB is suspected, refer Chapter 17 on Tuberculosis
Painful genital ulcer/s	Swab from base of the ulcer for - HSV 1/2 PCR	Collect with a premoistened sterile saline swab from the base of the lesion. Send the swab in a sterile container with VTM Transport at +4 to 8^{o}C for PCR and for culture at RT
	Bacterial culture	
	Blood/ serum for - HSV 1/2 IgM and IgG antibody test	
Painless genital ulcer	Exudate for - Dark field microscopy for *Treponema pallidum*	Collect the squeezed discharge from the ulcer base on to a cover slip and place it on a slide for microscopy examination
	Blood/ serum for - VDRL (nonspecific) *Treponema*-specific serology	TPPA and IgM-ELISA are *Treponema pallidum*-specific antibody tests
PID/ pelvic abscess/ tubo-ovarian abscess Extrapulmonary TB		Refer Chapter 12 on Intra-abdominal and Pelvic Infections and Chapter 17 on TB for detailed information

CHAPTER 5

Infection in the Immunocompromised Host

Note:

- ❖ Refer Chapter 19 on Specimen Collection and Transport for further information
- ❖ Blood culture and urine culture should be obtained in patients with unknown source of infection at admission.

Clinical syndrome	Microbiology investigations	Special instructions
Pneumonia	Expectorated sputum/ ET secretion/ BAL/ pleural aspirate for - Bacterial culture	Tests should be selected depending on the clinical and radiological evaluation BAL when obtained consider testing for bacteriological, fungal and/ or virological tests as it cannot be repeated frequently
	Blood culture	
	Blood / serum for - Bacterial Multiplex PCR	
	Nasopharyngeal aspirates/ BAL/ induced sputum for - Microscopy AFB	Refer Chapter17 on Tuberculosis
	NAAT for MTB Mycobacterial culture	
If fungal aetiology is suspected	Good quality sputum/ induced sputum/ BAL for - Direct microscopy for fungi /Toluidine stain for PJ Fungal culture	Refer Chapter 19 - Mycology for further information
	Pneumocystis jirovecii PCR	PJ-PCR available in university and private sector laboratories
	Blood/ serum for - *Aspergillus* galactomannan test Cryptococcal antigen test *Candida* mannan/antimannan test	Tests are available at the Mycology reference laboratory, MRI
If viral aetiology is suspected	Nasopharyngeal aspirates/ NP swabs/ induced sputum/ throat swabs in VTM for - PCR *Influenza* A/B and COVID-19	Consider rapid antigen tests for *Influenza* A/B and COVID-19, if PCR facilities are not available
	PCR for respiratory viruses and atypical pathogens	Consider quantitative PCR to monitor patients with viral infections e.g. CMV
Suspected parasitological aetiology	Sputum/ BAL for - Direct smear, culture and PCR for strongyloidiasis	Multiple specimens should be tested on consecutive days

Clinical syndrome	Microbiology investigations	Special instructions
Lung abscess / Empyema	Blood culture Aspirated pus/ fluid for - Bacterial culture Direct microscopy for fungi Fungal culture Blood/ serum - *Candida* mannan test/ *Candida* antimannan test Blood for - Melioidosis antibody test	Available at the Department of Microbiology, Faculty of Medicine, University of Colombo
	Aspirated pus/ fluid for - Mycobacterial culture NAAT for MTB	If TB is suspected, refer Chapter 17 on Tuberculosis
Urinary tract infection	Urine for - Bacterial and fungal culture Mycobacterial culture Direct microscopy for fungi Urine full report Urine / blood for - PCR for BK virus, adenovirus	If STI is suspected refer Chapter 4 on Genital Infections
Intra-abdominal infection/ peritonitis	Blood culture Ascitic fluid/ biopsy/ tissue from suspicious lesions for - Bacterial culture	
If fungal infection suspected	Fungal culture Direct microscopy for fungi Blood for - *Candida* mannan test / *Candida* antimannan test	Histopathology will be useful in fungal infections For further information, contact mycologist/ microbiologist
If TB suspected	Ascitic fluid/ biopsy/ tissue from suspicious lesions for - Mycobacterial culture NAAT for MTB	Refer Chapter 17 on Tuberculosis

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Neutropenic enterocolitis	Blood for - Bacterial and fungal cultures	
Diarrhoea	Stool full report	
	Stool/ faecal swab for - Bacterial culture	Transport stool specimen in RT for bacterial culture
Antibiotic-associated diarrhoea	Stool for - GDH antigen assay *Clostridium difficile* toxin A/B assay NAAT for toxin detection	Collect stools into dry, clean, screw capped container and transport at +2 to 8ºC in a cool box Check availability from Bacteriology II, MRI
If viral infection is suspected	Colonic biopsy for CMV PCR	Transport specimens for PCR and antigen detection at +2 to 8ºC in cool box
	Stool for - Rota virus antigen detection PCR for diarrhoeagenic viruses (rotavirus/ norovirus/ adenovirus/ sapovirus/ astrovirus etc.)	
	Hepatitis E PCR	Check availability before specimen collection
If parasitological aetiology is suspected	Stool microscopy Modified acid-fast stain for - • Cryptosporidiosis • Cystoisosporiasis • Cyclosporiasis	Fresh sample of stool in a dry, clean, wide-mouthed, leak proof container. Do not contaminate with urine If the first sample is negative, examination of 3 or more samples collected on separate days is recommended
	Stool for - Direct microscopy/ culture/ PCR for strongyloidiasis	Multiple samples on consecutive days should be collected Discuss with microbiology laboratory prior to sample collection
Central Nervous System infections Suspected cryptococcal meningitis	CSF for - Direct microscopy India ink stain Fungal culture	For routine ***bacterial and viral diagnosis***, refer Chapter 7 on Infections of the CNS

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Blood/ CSF for - *Cryptococcus* capsular antigen detection	
If viral infection is suspected - CMV, EBV, HHV6, HSV, VZV, JCV	CSF for - Viral Multiplex and Singleplex PCR	Collect CSF into sterile screw capped plastic tube and transport immediately to the laboratory at +4 to 8^{o}C in a cool box Check availability before sample collection. Contact virologist/ microbiologist
In addition, consider as appropriate: JE, dengue, measles, mumps	CSF/ EDTA blood for – IgM viral antibody tests for JE, dengue, measles, mumps	
	IgG antibody titre for SSPE	
Suspected Toxoplasmosis	Blood/ serum/ CSF for - *T. gondii* specific IgM & IgG antibody test	Paired samples should be collected 4-6 weeks apart to identify seroconversion or rising titre For IgM antibodies, collect the sample on day 5 of illness. IgG antibodies, usually appear after the 2nd week of illness. Available at the Department of Parasitology, MRI
	Blood/ serum/ CSF for - *Toxoplasma* specific PCR	Perform in few laboratories in universities and private sector. Check availability
Skin and soft tissue infection	Blood culture	Collect two blood cultures prior to initiating antibiotic therapy. Optimal time of collection is during a temperature spike
Ecthyma gangrenosum	Blister fluid/ if no fluid, swab the tissue underlying eschar for - Microscopy Gram stain Bacterial culture	
Cutaneous mucormycosis	Skin biopsy/ necrotic and pale tissue/ blister fluid for - Direct microscopy for fungi Fungal culture	Histopathology is useful in fungal aetiology
Viral aetiology HZV, VZV, HSV reactivation, monkey pox	EDTA blood/ blister fluid/ swab from base of the ulcer for - HZV/VZV/ HSV PCR	
	Vesicular fluid/ swab from base of the ulcer/ scab for - MPXV PCR	Contact virologist/ microbiologist for further information before sample collection

CHAPTER 6

Infections of the Cardiovascular System

Note:

❖ Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	**Microbiology investigation**	**Special instructions**
Infective Endocarditis (IE) (native and prosthetic valve)	Blood culture	In acute IE, draw three peripheral venous blood cultures 30 minutes apart from three separate venipunctures to demonstrate continuous bacteremia For subacute IE, draw minimum of three peripheral venous blood cultures over 24 hours before antibiotics Initiation of antibiotics should not be delayed in acutely ill patients. Do not delay sampling to coincide with peaks of fever In *Staphylococcus aureus* bacteremia or candidemia, repeat the blood cultures 48-72 hours after initiating antibiotics and every 48 hours until cultures are negative For certain organisms causing IE antibiotic MIC testing is required. Discuss with the microbiologist
	Vegetations/ explanted prosthetic valves for bacterial culture	
	Blood for fungal culture	Fungal endocarditis must be considered in the clinical setting of culture-negative that fails to respond to appropriate antibiotic therapy If fungal infection is suspected, collect blood into fungal blood culture bottles Contact mycologist/ microbiologist
	Vegetations/ explanted prosthetic valve for - Fungal culture Tests for fungal markers	
	Molecular and serological assays: Blood/ serum for - *Brucella* PCR & serology *L. pneumophila* PCR *Mycoplasma* antibody test	Consider testing if bacterial cultures are negative and/ or patient is not responding to treatment Available at the NRL, Department of Bacteriology, MRI. Contact microbiologist for further information

Clinical syndrome	**Microbiologyinvestigation**	**Special instructions**
Cardiac Implantable Electronic Device (CIED) related endocarditis	Blood culture	Minimum three blood cultures should be collected before initiation of empiric antibiotic therapy In patients with positive blood culture, repeat the blood culture 48-72 hours after initiating antibiotics and every 48 hours until cultures are negative
	Pocket-site tissue/ pus for - Bacterial culture	Percutaneous aspiration of the generator pocket should not be performed for diagnostic evaluation of CIED infection
	If CIED explanted, lead tip and generator-pocket tissue for bacterial culture	The explanted devices and leads could be submitted to the microbiology laboratory in a sterile container with sterile normal saline for culture
	Vegetation/ cardiac tissue/ sewing ring/ arterial embolus for - Bacterial culture	Immediately transport to the laboratory at room temperature

CHAPTER 7

Infections of the Central Nervous System

Note:

❖ Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Meningitis/ encephalitis/ meningoencephalitis	Blood culture	Preferably collect blood and CSF before antimicrobial therapy
	CSF for - Full report	CSF full report including cell count, protein and sugar are useful
	Bacterial culture	Send the 2nd or 3rd CSF bottle for bacterial culture or molecular tests as they have less potential for skin contamination Do not refrigerate specimens for bacterial culture as some organisms are inhibited in low temperatures
	CSF bacterial antigen detection test	Sensitivity and specificity of antigen detection is lower than PCR
	CSF/ blood for - Bacterial multiplex PCR (*S. pneumoniae, H. influenzae, N.meningitidis*)	Test is performed at NRL Clinical Microbiology, MRI
If viral infection is suspected HSV, VZV, dengue, *Influenza*, COVID-19, JE, measles, *mumps, Nipah*, *Chikungunya, West-Nile virus, Zika* virus	CSF for - Viral Multiplex and Singleplex PCR	If CSF is not available, testing with blood will only give supplementary evidence for the presence of infection
	CSF/ EDTA blood for - IgM and IgG antibody test for JE, dengue, measles	
If TB is suspected	CSF for - NAAT for MTB Mycobacterial culture	If immunocompromised or specific infection is suspected, refer Chapter 5

Clinical syndrome	**Microbiology investigations**	**Special instructions**
If fungal meningitis is suspected	CSF for - Direct microscopy for fungi, India ink stain Fungal culture	
Cryptococcus spp.	Blood/ serum/ CSF for - *Cryptococcus* capsular antigen detection	
Candida spp.	Blood/ serum for - *Candida* mannan antigen and anti-mannan antibody	In adults, *Candida* meningitis is often a post-neurosurgical complication especially after CSF shunt placement
Aspergillus spp.	Blood/ serum for - galactomannan antigen test	
Hospital-associated ventriculitis or meningitis (post-neurosurgery or head trauma, shunt/ drain infections)	Blood culture	Blood should be collected if the shunt terminates in a vascular space
	CSF from shunt/ drain/ reservoir for - Bacterial culture Fungal culture Direct microscopy for fungi	CSF should be collected from the sampling port of an EVD or lumbar drain, and not from the bag Culture should not be performed after removal of the shunt or drain, unless the patient has symptoms of CNS infection
Brain abscess/ Subdural empyema/ Epidural abscess	Pus/ aspirates/ tissue for - Bacterial culture	Do not refrigerate specimens for bacterial culture
	Blood culture	Do anaerobic cultures if facilities are available
If fungal infection suspected	Pus/ aspirate/ CSF for - Direct microscopy for fungi, India ink stain Fungal culture	Suspect fungal aetiology in immunocompromised patients or if features of fungal infection present radiologically or during surgery
Cryptococcus spp	CSF/ blood for *Cryptococcus* capsular antigen detection	
Candida spp.	Blood/ serum for - *Candida* mannan antigen and anti-mannan antibody tests	
Aspergillus spp	*Aspergillus* galactomannan antigen test	Useful in diagnosing *Aspergillus* infection
Mucorales Melanized fungi	Necrotic and pale material/ tissue/ pus for - Fungal culture	Mucorales are common environment contaminants. Isolation should be interpreted with caution in the absence of positive direct microscopy Histopathology of necrotic tissue is useful

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Chronic meningitis (symptoms for ≥4 weeks)		
TB meningitis	CSF for - NAAT for MTB Mycobacterial culture	Refer Chapter 17 Tuberculosis for further information
Cryptococcal meningitis	CSF for - Direct microscopy for fungi, India ink stain Fungal culture Blood/ serum / CSF for - Cryptococcal antigen detection	
Histoplasma capsulatum	Urine for - *Histoplasma* antigen test	
Parasitic meningo-encephalitis *Acanthamoeba* spp., *Naegleria fowleri, Angiostrongylus cantonensis*	CSF for - Direct microscopy PCR	Parasitic infections are rare Contact laboratory prior to sample collection to check test availability Refer Chapter 19 - Parasitology
Suspected human rabies infection Antemortem specimens	CSF and blood for - Rabies virus antibody test by tissue culture	Before sample collection, contact the Department of Rabies, MRI Both CSF and blood should be sent simultaneously for concurrent testing
	Pooled saliva for - Rabies virus PCR	All specimens should be maintained and transported at +4-8ºC in a leak- proof container
Postmortem specimens	Postmortem brain of the patient for - Direct Fluorescent antibody test (DFAT)	Send the whole brain to the Rabies reference laboratory, MRI. Do not add any fixatives, including normal saline Refer Chapter 19 - Virology

CHAPTER 8

Infections of the Ear, Nose, and Throat

Note:

- Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Otitis externa	Ear pus/ discharge/ swab for - Bacterial culture	Cultures are essential in patients with severe, recurrent, chronic non-resolving otitis externa, post-surgical patients and otitis externa in immunocompromised patients
Otomycosis	Ear pus/ discharge/ swab for - Direct microscopy for fungi Fungal culture	Fungal aetiology should be suspected in patients with poor response to topical antibacterials or patients on topical steroids Send two ear swabs premoistened with sterile normal saline
Otitis media	Middle ear fluid/ aspirates/ pus for bacterial culture Ear swab from discharge for bacterial culture	Do not refrigerate the specimens for culture If the tympanic membrane is ruptured in patients with myringotomy tubes, collect the discharge on swabs directly after cleaning the ear canal
Mastoiditis with ruptured tympanic membrane and purulent discharge	Ear pus/ swab for - Bacterial culture	Do not refrigerate samples for culture Follow strict aseptic procedure while collecting the sample
with intact tympanic membrane	Ear pus/ swab collected at myringotomy for - Bacterial culture	Label specimen as pus when collected from myringotomy Since the volume of pus is minimal, a swab for culture is considered as an acceptable sample
at mastoidectomy	Bone pieces/ pus/ bone nibbles collected at surgery for - Bacterial cultures (aerobic and anaerobic)	Place the bone nibbles in a sterile screw capped container with sterile normal saline and/ or in anaerobic media. Transport to the laboratory as soon as possible. Contact microbiologist for further information
If tuberculosis is suspected	Ear discharge/ pus for - NAAT for MTB Mycobacterial culture	Histopathology is useful in tuberculosis Refer Chapter 17 on Tuberculosis for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Sinusitis and rhinosinusitis Acute or chronic bacterial sinusitis	Sinus aspirate/ antral washouts/ fluids and tissue obtained during FESS for bacterial culture	Swabs are not recommended Nasal swabs from blind swabbing or purulent nasal secretions are unreliable in the diagnosis
Acute fungal sinusitis/fungal sinusitis chronic or allergic	Sinus aspirate/ sinus washouts/ antral washouts/ fluid and tissue obtained during FESS for - Direct Microscopy for fungi Fungal culture	Histopathology from tissue obtained during Functional Endoscopic Sinus Surgery (FESS) is useful for the diagnosis Refer Chapter 19 - on Mycology Specimens at FESS should be sent in sterile screw capped container in sterile normal saline at RT for bacterial and fungal cultures
Acute pharyngitis /tonsillitis		Acute tonsillitis/ pharyngitis mostly viral in origin. Cultures are not routinely indicated
If a recurrent bacterial infection is suspected	Throat swab for - Bacterial culture	
If Infectious mononucleosis is suspected	Blood/ serum for - EBV-specific antibody detection tests: EBV VCA IgM, EBV VCA IgG, EBNA IgG	Usually, a clinical diagnosis unless it is an atypical presentation Blood picture will be helpful
Suspected *Diphtheria*	Nasopharyngeal secretions/ NP swab/ nasal swab/ mucosal lesions for - *Diphtheria* culture	Culture facilities are routinely not available for *C. diphtheriae* Contact microbiologist before sample collection
Epiglottitis	Blood culture Epiglottic swab for - Bacterial culture **(only in intubated patients)**	Throat swabs are contraindicated in patients suspected of acute epiglottitis as it may precipitate upper airway obstruction

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Peritonsillar/ retropharyngeal/ parapharyngeal abscess	Aspirates/ drained pus from peritonsillar/ parapharyngeal abscess for bacterial culture (aerobic and anaerobic)	Throat swab is not an acceptable sample Send anaerobic specimens in cooked meat broth or other anaerobic media Collect specimen aseptically into a sterile screw capped container and transport to the laboratory as soon as possible
If TB is suspected	Aspirates/ drained pus from peritonsillar/ parapharyngeal abscesses for - Mycobacterial culture NAAT for MTB	Refer Chapter 17 on Tuberculosis
Suppurative jugular thrombophlebitis (Lemierre's syndrome)	Blood culture Throat swab/ pus for - Bacterial culture (aerobic and anaerobic)	Blood should be collected into an anaerobic blood culture bottle when facilities are available The diagnosis should be suspected clinically and inform the laboratory to select suitable tests and methods Contact consultant microbiologist before sample collection
Oropharyngeal candidiasis/ oral thrush	Oral/ throat secretions/ pus/ scrapings of mucosal lesions for - Direct microscopy for fungi Fungal culture	Testing for candidiasis is not routinely done Collect two swabs and transport to laboratory at RT If not responding to antifungal treatment, isolates could be sent for species identification and antifungal susceptibility testing to mycology reference laboratory, MRI

CHAPTER 9

Infections of the Eye

Note:

- ❖ Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Conjunctivitis	Conjunctival swab for - Bacterial culture	Microbiological investigations are considered in an outbreak and in special situations when an aetiological diagnosis is required e.g. in neonates, post-surgical infection, immunocompromised patients Two separate eye swabs should be obtained. This will help to compare the isolated pathogen with normal flora Sterile Dacron, rayon or nylon flocked swabs should be used for sample collection for PCR. Place swabs in VTM aseptically and transport to the laboratory at +4 to 8°C in cool box
	Conjunctival swab for - Virus PCR	
Ophthalmia neonatorum is suspected	Conjunctival swab/ discharge for - Microscopy Gram stain Bacterial culture Gonococcal culture	In neonatal conjunctivitis, GC and *Chlamydia* should be suspected in high-risk mothers For GC culture, prepare slides and inoculate special media plates at bedside If culture facilities are not available, send swabs in Amies transport medium at RT to reach the testing laboratory within 24-48 hours
	Chlamydia/ Gonococcal PCR HSV 1/2 PCR	
Keratitis	Corneal scrapings/ corneal button for bacterial culture	Specimens should be collected by an ophthalmologist If the specimen is minute, inoculate the culture plates and prepare slides at the site of collection or send in a sterile screw capped container with sterile normal saline or brain heart infusion broth (BHI) at RT Contact microbiology laboratory for microscopy slides and culture plates Bedside preparation:

Clinical syndrome	Microbiology investigations	Special instructions
		First inoculate the sample in culture medium and subsequently make the smear on the slide Mark a circle on the slide and place the specimen on the reverse side of the circle to locate the site of smear easily
	Corneal scrapings for viral PCR	Place scrapings in VTM and transport at +4 to 8°C in cool box for virological studies
Fungal keratitis Keratomycosis	Corneal scrapings/ corneal button for - Direct microscopy for fungi Fungal culture	First inoculate the specimen in the culture medium and make the smear on the slide for microscopy subsequently. Mark a circle on the slide and place the specimen on the reverse side of the circle to locate the site of smear easily Contact microbiology laboratory for microscopy slides and culture plates or send the specimen in sterile screw capped container with 2mL sterile saline
Contact Lens-associated keratitis	Contact lens/ lens solution from bottle/ lens cases for - Bacterial culture Direct microscopy for fungi Fungal culture	Should be collected aseptically Send lenses in sterile container with sterile normal saline or with lens solution in RT
Refractory keratitis	Corneal button/scrapings for - Bacterial culture Direct microscopy for fungi Fungal culture	Contact microbiologist before sample collection Parasitological aetiology should be considered
Retinitis/acute retinal necrosis / progressive outer-retinal necrosis	Aqueous/ vitreous humour for CMV, HSV,VZV PCR	Specimen is obtained by an ophthalmologist Collect into a sterile plastic container or in a sealed syringe and transport at + 4 to 8°C in cool box
Endophthalmitis / Uveitis	Intraocular pus/ fluid for - Bacterial culture Fungal culture Direct microscopy for fungi	Specimen should be sent in the syringe used for collection. Recap the needle and secure the plunge with plasters for safety. Diagnosis of endogenous fungal infection to be considered in patients with debilitating illness
Orbital cellulitis	Pus/ sinus aspirates/ swab for - Bacterial culture Direct microscopy for fungi Fungal culture Blood culture	Collect purulent material from nose and sinuses into a sterile container and transport at RT Although low yield in blood culture, should be obtained prior to antibiotic therapy

CHAPTER 10

Infections of the Respiratory Tract

Note:

- ❖ Refer Chapter 19 on Specimen Collection and Transport for further details
- ❖ First morning expectorated sputum is always best for bacterial culture
- ❖ In the immunocompromised host, a broad diagnostic approach is indicated

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Upper respiratory tract infections Suspected bacterial / viral infections	Cultures are not indicated routinely as most infections are viral in origin and self-limiting	Refer Chapter 8 on Infections of the Ear, Nose, Throat
Lower respiratory tract infections Acute bronchitis	Nasopharyngeal (NP) aspirates/ NP swabs/ throat swabs in VTM for - Rapid antigen tests - *Influenza* A/B & COVID-19 PCR for *Influenza* A/B and COVID-19 PCR for other respiratory viruses and atypical bacteria	Nasopharyngeal aspirates and NP swabs are more suitable than throat swabs Due to sub-optimal performance characteristics of the antigen test, it is recommended to correlate test results with clinical and other relevant laboratory findings for patient management This test is done only in outbreaks
Suspected Whooping cough/ pertussis	Nasopharyngeal aspirates/ NP swabs/ throat swabs for - Multiplex PCR for *Bordetella* spp.	Test performed at the NRL Clinical Microbiology, MRI For PCR and antigen tests, store and transport specimens at +4 to 8°C in a cool box
Infective exacerbation of COPD / bronchiectasis	Sputum/ bronchoscopy specimens for - Bacterial culture	Bronchoscopy specimens are preferred
Suspected viral infection	Nasopharyngeal aspirates/ NP swabs/ throat swabs in VTM for - Rapid antigen test for *Influenza* A/B & COVID-19	Contact virologist/ microbiologist Please see above for the comment

Clinical syndrome	**Microbiology investigation**	**Special instructions**
	PCR *Influenza* A/B and COVID-19 PCR for other respiratory viruses and atypical bacteria	
	Blood/ serum for - *Mycoplasma* antibody test	Test available at few centers including NRL Clinical Microbiology, MRI and SJGH, Nugegoda
Community acquired pneumonia	Blood culture	
	Sputum/ bronchoscopy specimens/ endotracheal aspirates in ventilated patients for - Bacterial culture	Poor-quality respiratory specimens could provide misleading results
	Urine antigen test for - *Streptococcus pneumoniae*	Available in few laboratories
	Blood/ serum for multiplex PCR (*S. pneumoniae, H. influenzae, N. meningitidis)*	Test available at the NRL Clinical Microbiology, MRI
Suspected atypical pneumonia	Urine antigen test for - *Legionella*	Available in few laboratories
	Respiratory specimens for - *Legionella pneumophila* PCR	Test performed at the NRL Clinical Microbiology, MRI
	Blood/ serum for - *Mycoplasma* antibody test	Test available at few centers including NRL Clinical Microbiology, MRI and SJGH, Nugegoda
Suspected viral infection	NPA/NP swabs/ induced sputum/ throat swabs in VTM for - PCR *Influenza* A/B and COVID-19 PCR for respiratory viruses and atypical pathogens	Contact consultant virologist/ microbiologist for further information If not responding to treatment, consider testing for mycobacterial infection Refer Chapter 17 on Tuberculosis
Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia	Endotracheal aspirates/ bronchoscopy specimens/ tracheal aspirate/ sputum for - Bacterial culture Blood culture	

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Empyema	Aspirated pus/ tissue/ fluid for - Bacterial culture Blood culture	Culture from intercostal drain is not recommended due to colonizing flora If imaging studies are suggestive, consider testing for mycobacterial infection
Lung abscess	Aspirated pus/ tissue/ biopsy/ sputum for - Bacterial culture Blood culture	If imaging studies are suggestive, consider testing for mycobacterial infection
Pulmonary tuberculosis		Refer Chapter 17 on Tuberculosis
Suspected fungal lung infection Acute invasive fungal infection	Bronchoscopy specimens/ lung biopsy/ tissue/ sputum/ tracheal aspirates for - Direct microscopy for fungi Fungal culture	Bronchoscopy specimens and lung biopsy tissue is preferred If other fungal aetiology is suspected, contact mycologist or microbiologist
Suspected chronic cavitary pulmonary aspergillosis (CCPA)/ mycetoma/ aspergilloma	Serum/ BAL for *Aspergillus* galactomannan antigen test Bronchoscopy specimens/ lung biopsy tissue/ sputum/ tracheal aspirates for - Direct microscopy for fungi Fungal culture Serum/ BAL for - *Aspergillus* galactomannan antigen test	
Suspected Allergic bronchopulmonary aspergillosis (ABPA)	*Aspergillus*-specific IgG antibody test Total IgE antibody test *Aspergillus* specific IgE antibody test *Aspergillus* precipitin test	Contact mycologist/ microbiologist Send specimens to mycology reference laboratory, MRI
Suspected *Pneumocystis jirovecii* (PJ) pneumonia	Good quality sputum/ induced sputum/ BAL for - Direct microscopy Toluidine stain for PJ *Pneumocystis jirovecii* PCR	Done in few laboratories in universities and private sector

CHAPTER 11

Infective Hepatitis

Clinical syndrome	Microbiology investigations	Special instructions
Acute infective hepatitis		Completed request form with the clinical presentation is essential
For routine screening	Blood/ serum for -	Contact virologist/ microbiologist to select the suitable investigations and markers depending on clinical information
Hepatitis A	Hepatitis A IgM antibody test	
Hepatitis B	Hepatitis B surface antigen (HBsAg) test	First line investigation is HBsAg detection
	Hepatitis B serology profile testing	
	EDTA blood for - Hepatitis B virus PCR	
Hepatitis C	EDTA blood for - Hepatitis C virus PCR	
	Blood/ serum for - Hepatitis C antigen + antibody combo test	
Hepatitis E	Blood/ serum for - Hepatitis E IgM antibody test	Check availability of tests before specimen collection
Chronic infective hepatitis		
For routine screening	Blood/ serum for - Hepatitis B surface antigen (HBsAg) test Hepatitis C antibody test	First line investigation is HBsAg for the detection of causative agent
	EDTA blood for - Hepatitis B/ C virus PCR	Contact virologist/ microbiologist to select suitable investigations and markers
Other infective hepatitis in special patient population		Discuss with virologist/ microbiologist Refer Chapter 5 on Infections in Immunocompromised Host

CHAPTER 12

Intra-abdominal and Pelvic Infections

Note:

- Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Sepsis/septic shock + acute abdomen	Blood culture	Ideally before antibiotics. Consider anaerobic culture if facilities are available
	Pus/ aspirates/ peritoneal fluid for - Bacterial culture (aerobic/ anaerobic)	For aerobic cultures, collect into sterile containers with sterile normal saline For anaerobic cultures, use cooked meat broth or any other medium if facilities are available
If TB is suspected	NAAT for MTB Mycobacterial culture	Contact mycologist/ microbiologist for fungal cultures and fungal markers
Biliary sepsis (acute cholecystitis/ cholangitis / biliary obstruction)	Blood culture	Contact microbiologist for anaerobic blood cultures if required
	Bile/ pus for - Microscopy Gram stain Bacterial culture	Samples should be collected during percutaneous transhepatic cholangiography (PTC), endoscopic retrograde cholangiopancreatography (ERCP) or during surgery
	Stents in sterile saline for - Bacterial culture	
Acute appendicitis (in ruptured appendix or appendicular abscess)	Blood culture	
	Pus collected during surgery for - Bacterial culture	

Clinical syndrome	**Microbiology investigation**	**Special instructions**
Liver abscess	Blood culture	Collect specimens for culture before antibiotic administration
Pyogenic liver abscess	Pus from radiologically-guided, diagnostic or therapeutic aspiration for - Bacterial culture (aerobic and anaerobic)	Collect pus for anaerobic cultures into an anaerobic transport media (cooked meat broth). If it is aspirated, send in a sealed syringe taking safety precautions
± splenic abscess	Blood/ serum for - Melioidosis antibody test	Performed at the Department of Microbiology, Faculty of Medicine, University of Colombo
Suspected amoebic liver abscess	Radiologically-guided aspirated pus for - Direct microscopy wet mount	Preferably the terminal part of the aspirate should reach the lab within one hour of collection. Do not refrigerate.
	PCR	Inform the laboratory before specimen collection and check availability of PCR Store and transport specimens at +2 to 8°C in a cool box
Spontaneous bacterial/ secondary peritonitis	Blood culture Peritoneal fluid for - Cell count Bacterial culture	Send in a sterile screw capped container and transport at RT If fungal aetiology or TB is suspected, contact mycologist/ microbiologist
Continuous ambulatory peritoneal dialysis (CAPD) peritonitis	PD effluent in a sterile screw capped container for - Cell count Bacterial culture	Infuse 1L of dialysate and allow to dwell for a minimum of 2 hours. Then drain 50mL of the dialysate for culture Results should be interpreted carefully because of the presence of colonizing flora
	Discharge/ PD effluent/ swab from exit site for - Bacterial culture	Exit site infection may follow catheter-associated peritonitis. Look for the presence of the same organism in the exit site/ tunnel and the peritoneal fluid
	Blood culture	Collect blood for culture if the patient is septic or on immunosuppression
	Peritoneal dialysis effluent for Fungal culture	If bacterial culture is negative and evidence of peritonitis is present, samples should be sent to Mycology reference laboratory, MRI
	Mycobacterial culture NAAT for MTB	If TB is suspected Contact mycologist/ microbiologist for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Pelvic Inflammatory Disease	Blood culture HVS for - Microscopy Gram stain Bacterial culture Endocervical swab for - Microscopy Gram stain Gonococcal culture PCR for GC /*Chlamydia*	Blood cultures in systemically ill patients Two separate swabs are required Slides and plates can be obtained from the microbiology laboratory for bedside preparation in suspected gonococcal infections If culture facilities are not available, transport in Amies transport medium at RT, to reach the testing laboratory within 24-48 hours Refer to a venereologist or the nearest STDclinic
Pelvic abscess/ Tubo-ovarian abscess If other aetiology suspected	Blood culture Pus/ tissue for - Bacterial culture Pus/ tissue for - NAAT for MTB Mycobacterial culture Pus/ tissue for - Microscopy Gram stain Gonococcal culture GC/*Chlamydia* PCR	In acutely ill patients blood culture should be done Do not refrigerate samples obtained for culture Refer Chapter 17 on Tuberculosis If STI is suspected, contact microbiologist/ venereologist before sample collection Pus samples for gonococcal cultures should be collected into Amies transport medium

CHAPTER 13

Infections of the Musculoskeletal System

Note:

❖ Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Acute bacterial osteomyelitis	Blood culture Bone tissue/ soft tissue/ pus for - Bacterial culture Types of specimens; Radiologically obtained percutaneous bone biopsies, intra-operative bone biopsies & deep soft tissue, bone and soft tissues around prosthetic devices (fracture fixation plate, nail), radiologically-guided aspirated pus surrounding the infected bone	Blood culture should be obtained in all children with acute osteomyelitis, suspected adult hematogenous osteomyelitis in patients with sickle cell disease, on hemodialysis and in intravenous drug users Pus swabs or superficial wound swabs are not suitable Send tissue specimens in sterile normal saline to prevent drying Bone biopsies for histopathology could give supportive evidence
Chronic osteomyelitis In vertebral osteomyelitis, bone TB is suspected Brucellosis (travel to endemic area /occupational risk of brucellosis	Bone tissue biopsy/ aspirated pus for - Bacterial culture NAAT for MTB Mycobacterial culture Blood culture for *Brucella* Blood/ serum/ sterile fluid for *Brucella* PCR Bone marrow/ synovial fluid aspirates for - *Brucella* culture *Brucella* serology	Samples from the sinus tract and superficial swabs are discouraged due to colonizing organisms Bone biopsies for histopathology is useful Contact microbiologist before collection of specimens for special tests such as TB, *Brucella* etc. Automated blood culture bottles are recommended Test performed at the NRL Clinical Microbiology, MRI Acute and convalescent blood (3-5mL) 14 days apart. Should sent in plain sterile tube for *Brucella* antibody test Test available at Bacteriology II, MRI

Clinical syndrome	Microbiology investigations	Special instructions
Septic arthritis	Synovial fluid obtained via arthrotomy/ arthrocentesis for - Synovial fluid full report Synovial fluid culture Blood culture	If the initial tests are negative, consider further testing for TB, anaerobic and/or gonococcal infections Superficial swabs from skin lesions are not recommended In acute infection or sepsis, obtain blood for culture
Prosthetic joint infection If fungal aetiology is suspected	Blood culture Synovial fluid/ percutaneous joint aspiration for - Synovial fluid full report Bacterial culture Synovial fluid/ tissue/ periprosthetic tissue/ bone tissue/ intra-operative peri-prosthetic tissue/ bone fragments for - Bacterial culture Direct Microscopy for fungi Fungal culture	Indicated in acute infection If no features of systemic infection, antibiotics should be delayed until sampling Minimum 3 (or optimally 5-6) intraoperative tissue samples should be obtained Before collecting intraoperative specimens for culture, keep the patient antibiotic-free for at least 14 days Superficial swabs from skin lesions are not recommended
Paraspinal/ epidural abscess/Potts disease/ psoas abscess **Pyomyositis**	Percutaneous aspirates/ pus/ tissue/ bone biopsy (radiologically-guided) for - Bacterial culture Percutaneous aspirate/ pus/ tissue/ bone biopsy (radiologically guided) for - Mycobacterial culture NAAT for MTB Blood culture Guided pus/ aspirates for - Bacterial culture	 Blood culture should be obtained if the patient is systemically ill

CHAPTER 14

Pyrexia of Unknown Origin

Note:

- ❖ For further information, refer the following chapters:
  - Chapter 19 on Specimen Collection and Transport in bacteriology, virology, mycology and parasitology
  - Chapter 17 on Tuberculosis
  - Chapter 6 on Infections in immunocompromised host
- ❖ Select relevant specimens and investigations after obtaining patient's history including present illness, recent and past clinical information, occupation relevant to the clinical presentation, physical examination and imaging studies
- ❖ Mention the suspected diagnosis in the request form, in addition to brief clinical history, travel, risk exposures to identify special laboratory requirements in sample processing and the interpretation of test results

Clinical syndrome	Microbiology investigations	Special instructions
Classical PUO - Temperature >101°F, twice or more, >3 weeks duration with no known immuno-compromised state Admission to hospital not essential (Select relevant investigations according to the clinical presentation, risk exposures, pets, travel (local/ overseas) etc.)	Blood culture	Obtain 2 blood cultures from separate sites >1 hour apart within 24 hours. If negative in 24-48 hours, repeat 2-3 blood cultures
	Sputum/ urine/ pus/ aspirates/ tissue/ CSF/ wound swab/ lower respiratory specimens/ bone marrow/ sterile fluid for - Bacterial culture	Bone marrow culture should be considered in patients suspected of enteric fever, TB, brucellosis or visceral leishmaniasis
	Stool culture	
	Thick & thin blood smear microscopy for malaria RDT for malaria antigen	
	Early morning expectorated sputum/ induced sputum/ BAL/ bronchial wash/ ET secretions for - Microscopy AFB	Specimens can be sent in <u>clean</u> screw capped container for AFB
	Lower respiratory specimens/ pus/ aspirates/ tissue/ bone marrow/ sterile fluid/ lymph node biopsy/ biopsy tissue of suspicious lesions for - Mycobacterial culture NAAT for MTB	Collect specimens into a <u>sterile</u> screw capped container for culture and NAAT
	Blood/ serum for - IGRA assay for TB	
	Blood/ serum for - Slide agglutination test for enteric fevers	This test has limited value in enteric fever endemic countries as positive result may indicate previous infection

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Blood for HIV antigen + antibody test	Send specimens to the nearest STD clinic
	Blood/ serum for - melioidosis antibody test	Performed at the Department of Microbiology, Faculty of Medicine, University of Colombo
	Blood/ serum for *Brucella* PCR	Performed at the NRL Clinical Microbiology, MRI
	Brucella serology	Performed at the Bacteriology II, MRI
	Blood/ serum for - Weil-Felix antibody test	Test has limited value due to low sensitivity and specificity
	Rickettsia immunofluorescence assay	Send 3mL blood in sterile plain tube. Test performed at the Department of Parasitology, Faculty of Medicine University of Kelaniya
	Rickettsia PCR	Check availability from Bacteriology II, MRI
Suspected fungal infections	Blood for fungal culture	Collect specimens prior to starting antifungals or at least just before the next dose, if the patient is already on antifungals
	BAL/ bone marrow/ lymph node/ biopsy tissue/ sterile fluid/ pus/ aspirates for - Direct microscopy for fungi Toluidine blue for PJ Fungal culture	Depending on clinical signs and symptoms consider relevant tests
	Serology for invasive fungal infections	Refer Chapter 19 - Mycology, serological assays
Suspected toxoplasmosis	Blood/ serum for - *T. gondii* specific IgM/ IgG antibody test by ELISA	Refer Chapter 19 - Parasitology Available at the Department of Parasitology, MRI
	T. gondii specific PCR	Available in few laboratories in universities and private sector
If viral aetiology is suspected	PCR for CMV, HSV Serology for EBV	Refer Chapter 19 - Virology
Nosocomial PUO -Temperature >101°F in a hospitalized patient in whom infection was not manifested or incubating on admission	Peripheral blood culture	Collect blood simultaneously and in similar volume
	Central line blood culture	
	Central line tip for culture	If central line infection is suspected, send a blood culture concurrently with the line tip
	Urine/ pus/ aspirate for - Bacterial/ fungal cultures	
	Sinus secretions for - Bacterial/ fungal cultures	Especially in patients with nasogastric tubes

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Thick and thin blood smear for microscopy	Important after blood transfusion and overseas travel
	RDT for malaria	Contact AMC
	Blood/ serum for dengue virus serology	Consider in hospitalized patients in an outbreak
If antibiotic-associated diarrhoea is suspected	Diarrhoeal stools for - GDH antigen assay *C. difficile* toxin A/B assay Nucleic Acid Amplification Test for toxin detection	Check availability from Bacteriology II, Department of Bacteriology, MRI
Neutropenic PUO Temperature of >101°F with absolute neutrophil count is < 500/µL	Blood culture	Minimum two blood cultures should be obtained
	Relevant specimen types after clinical examination; urine/ sputum/ pus/ wound aspirates/ swabs/ BAL for - Bacterial culture	Infections are commonly caused by endogenous flora
	Blood/ serum/ CSF for PCR *S. pneumoniae, H. influenzae, N. meningitidis*	Test performed at the NRL Clinical Microbiology, MRI
	Respiratory specimens for - *Legionella pneumophila* PCR	Test performed at the NRL Clinical Microbiology, MRI
	Blood for *Mycoplasma* antibody test	Test available in few laboratories including NRL Clinical Microbiology, MRI and SJGH, Nugegoda
Suspected invasive fungal infections	Relevant specimen types for - Direct microscopy for fungi Fungal culture	Refer Chapter 19 - Mycology
Aspergillosis	Blood/ BAL/ bronchial wash for *Aspergillus* galactomannan antigen test	
Candidiasis	Blood for - *Candida* mannan antigen and *Candida* antimannan antibody test	
Suspected viral infections HSV/ CMV/ EBV	CSF for - Viral Multiplex and Singleplex PCR	Refer Chapter 19 - Virology

Clinical syndrome	**Microbiology investigations**	**Special instructions**
HIV-associated PUO -Temperature of >101°F on several occasions over a period of >4weeks for outpatients and >3 days for hospitalized patients with HIV infection	Syphilis serology	Send specimens to the nearest STD clinic
	Test for opportunistic infections (OI)	The CD4 cell count (<200/μL) is a reliable indicator of the risk of acquiring opportunistic infections (OI)
	Good quality sputum/ induced sputum/ BAL for - Microscopy -Toluidine blue stain for *P. jirovecii*	Send in screw capped container at RT to Mycology reference laboratory, MRI
	CSF for - Microscopy India ink stain for *Cryptococcus* Fungal culture	Refer Chapter 19 - Mycology
If TB and other mycobacterial infections are suspected	Investigations for MTB and MAC	Refer Chapter 17 on Tuberculosis
	Stool/ faecal swab for - Bacterial culture	Fresh sample of stool in a dry, clean, wide-mouthed, leak-proof container. Do not contaminate stools with urine
If parasitological aetiology suspected	Stool for Modified acid-fast stain for the detection of - Cryptosporidiosis Cystoisosporiasis Cyclosporiasis	Contact microbiology laboratory before sample collection If the first sample is negative, examination of three or more samples collected on separate days is recommended
	Stool for - Direct microscopy/ culture/ PCR for strongyloidiasis	Refer Chapter 19 - Parasitology Multiple samples on consecutive days
Toxoplasmosis	Blood/serum/ CSF for - *T. gondii* specific IgM/ IgG antibody test	Paired blood samples should be tested 4-6 weeks apart
	Toxoplasma specific PCR	Performed only in few laboratories. Check availability
If viral infection is suspected	Serology/ PCR for - CMV/ VZV	Refer Chapter 19 - Virology Refer Chapter 5 - Infections of the Immunocompromised host
	Screening for viral hepatitis	Refer Chapter 11 - Infective hepatitis

CHAPTER 15

Sepsis and Septic Shock

Note:

- Refer to Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Community acquired sepsis and/or Septic shock (unknown origin)	Blood culture	Mention the relevant clinical history in the request form including occupational exposures and other risk factors
	Sputum/ urine/ pus/ aspirates/ tissue/ CSF/ wound swab/ HVS (-in postpartum sepsis/ septic abortion) for - Bacterial culture	Relevant specimens should be collected depending on the suspected organ or system affected
Suspected melioidosis	Blood/ serum for - Melioidosis antibody test	Test performed at the Department of Microbiology, Faculty of Medicine, University of Colombo
Suspected leptospirosis	Blood/ serum for - MAT, *Leptospira* PCR	Test performed at NRL leptospirosis, MRI Refer Chapter 19 - Leptospirosis
Suspected viral *Influenza*/ COVID-19	Nasopharyngeal aspirates/ NP swabs/ throat swabs in VTM for - Rapid antigen test for *Influenza* A/B, COVID-19	Due to sub-optimal performance characteristics of the antigen test, it is recommended to correlate test results with clinical and other relevant laboratory findings for patient management
	Influenza A/B, COVID-19 PCR	
Hospital-associated sepsis and/ or Septic shock (unknown origin)	Blood culture	Collect two blood samples from two different peripheral sites
	Relevant specimens for bacterial culture: sputum/ ET secretions/ BAL/ urine/ pus/ aspirates/ tissue/ pus swab/ CSF/ wound swab/ HVS (in postpartum sepsis/ septic abortions)	If anaerobic infection is suspected, contact microbiologist
If fungal infection is suspected	Blood for fungal culture	
	Blood/ serum for - *Candida* mannan antigen and antimannan antibody	

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Sepsis and/ or septic shock in patients with central line	Peripheral blood culture Central line blood culture Central line tip for bacterial culture	Collect blood from the central line and peripheral venous blood simultaneously If the central line is removed, send the line tip with a simultaneously collected peripheral venous blood
Sepsis and septic shock in immunocompromised host		Refer Chapter 5

CHAPTER 16

Skin and Soft Tissue Infections

Note:

- Refer Chapter 19 on Specimen Collection and Transport for further information

Clinical syndrome	Microbiology investigations	Special instructions
Cellulitis	Blood culture Pus/ blister fluid/ wound swab for - Bacterial culture	 Superficial swabs in the absence of skin breaks are not useful
Impetigo	Pus/ blister fluid/ exudate underneath the scab for - Microscopy Gram stain Bacterial culture	Impetigo is usually a clinical diagnosis Microbiological testing is done in outbreak situations, to identify methicillin-resistant *Staphylococcus aureus* (MRSA), if post-streptococcal glomerulonephritis is present, not responding to empiric therapy and in recurrent or persistent infection
Purulent skin & soft tissue infections cutaneous abscesses, furuncles,carbuncles **Necrotic skin and soft tissue infection** **Vesiculobullous skin lesions** Including HSV 1/2, VZV, monkey pox, enteroviruses	Aspirated pus/ pus swabs for - Bacterial culture Blood culture Aspirated pus/ pus swabs for - Bacterial culture Vesicular fluid/ crusts/ scabs/ swab from underneath ulcer for - HSV1/2, VZV, MPXV, EV PCR	Pus specimens are preferred than swabs If swabs are sent, two swabs are preferred for Gram stain and culture
Erysipelas	 Cutaneous pus/ blister fluid aspirates for - Bacterial culture Blood culture	In classic erysipelas microbiological testing is not required Superficial swabs in the absence of skin breaks are not useful Blood culture is useful in atypical cases when the diagnosis is in question, suspected metastatic infection or bacteremia, patients with prosthetic devices, immunocompromised patients with systemic illness

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Suspected skin lesions of leprosy	Slit Skin Smear (SSS) for - Microscopy AFB Skin and nerve biopsy for - Molecular studies and anti-microbial resistance test	Refer patient to the dermatology/ leprosy clinic or satellite clinics in hospitals Tests available at the Centre for Leprosy Diagnosis, Faculty of Medicine, University of Colombo
Surgical site infections	Pus/ tissue/ wound exudate/ aspirates/ swabs for - Bacterial culture Blood culture	Pus/ tissue specimens are preferred than swabs Swabs should be collected after cleaning the wound with sterile normal saline Blood culture only in severe infection with features of sepsis
Necrotizing fasciitis	Blood culture (aerobic and anaerobic) Pus/ exudate/ aspirates/ deep tissue for - Bacterial culture (aerobic and anaerobic)	Perform anaerobic cultures if facilities are available Pus or deep tissue samples obtained at the time of surgical debridement are preferred. Do not send necrotic or superficial tissue
Gas gangrene	Wound exudates/ aspirates/ tissues obtained from deep areas of the wound for - Microscopy Gram stain Bacterial culture (aerobic and anaerobic)	For special anaerobic medium, contact microbiology laboratory Histopathology is useful
Animal and human bite wound	Wound exudates/ tissue/ pus/ swabs for - Bacterial culture	
Burn / trauma wounds	Blood culture Exudates/ pus/ swabs/ tissue for bacterial culture	
Cutaneous leishmaniasis	Slit skin scraping microscopy Lesion aspirates for - microscopy/ culture/ PCR	Refer Chapter 19 - Parasitology Refer to a dermatologist and inform the AMC
Chronic subcutaneous lesions with sinuses suspected of mycetoma	Deep biopsy tissues/ granules/ sinus aspirates for - Bacterial culture (aerobic and anaerobic) Direct microscopy for fungi Fungal cultures	Send tissue samples in sterile normal saline to prevent drying For anaerobic cultures, use special anaerobic media if facilities available Contact microbiology laboratory for further information

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	Deep biopsy tissues/ granules for - Microscopy with special fungal stains	Histopathology will be useful
Superficial cutaneous fungal infections Tinea infection Cutaneous candidiasis	Skin scrapings from edge of the lesion for - Direct microscopy for fungi Fungal culture	*Candida* species are frequent colonizers of cutaneous lesions as they are skin commensal. Therefore, direct microscopy is more significant than isolation in culture
Pityriasis versicolor Malassezia Folliculitis Seborrheic dermatitis	Skin scrapings from the lesion or sellotape mounts on a glass slide for - Direct microscopy for fungi	
Subcutaneous fungal infections Chromoblastomycosis	Deep surgical biopsy tissue / skin scrapings for - Direct microscopy for fungi Fungal culture	Do not send swabs
Sporotrichosis	Pus/ tissue/ skin biopsy for - Direct microscopy for fungi Fungal culture	
Fungal nail infections Dermatophytes/ *Candida* and other moulds	Nail scrapings/ nail clippings/ sub-ungual debris/ material from swollen periungual nail wall/ pus under the nail for - Direct microscopy for fungi Fungal culture	Isolation of *Candida* species is seldom significant unless the organism is seen in direct microscopy
Fungal hair infections Dermatophyte infection/ kerion/ black & white piedra	Plucked hair roots/ contents of follicles/ skin scales/ black or white nodules on hair/ hair fragments/ epilateral hair/ pus or swab from infected kerion/ pus crusts for - Direct microscopy for fungi Fungal culture	Hair without roots or nodules are unacceptable

CHAPTER 17

Tuberculosis

Note:

- Further information is available in the 'National Manual of Tuberculosis Control', published by the National Programme for Tuberculosis Control and Chest Diseases (NPTCCD), MoH
- When obtaining samples, adhere to strict infection control measures
- Xpert MTB/RIF Ultra and Truenat MTB are automated nucleic acid amplification tests (NAAT), which are WHO-recommended rapid diagnostics (WRDs) for early detection of TB and rifampicin resistance
- Automated liquid TB cultures (MGIT) will be performed depending on the request, type of specimen and the availability of the test. Sample collection is the same as for solid TB cultures.

Clinical syndrome	Microbiological investigations	Special instructions
Adult Pulmonary TB	Early morning expectorated sputum/ induced sputum/ BAL/ bronchial wash/ ET secretions for - Microscopy AFB	Containers could be obtained from the nearest chest clinic or microbiology laboratory Clean plain container is adequate for microscopy AFB
	Sputum/induced sputum/ BAL/ bronchial wash/ ET secretions for - NAAT for MTB Mycobacterial culture	For mycobacterial culture and molecular studies, specimen should be collected into sterile wide-mouthed, transparent, screw capped container Transport as early as possible. If delay store at +2 to 8ºC Three early-morning expectorated sputum 2-3mL in three consecutive days are preferred. Patient should be advised to collect sputum by vigorous coughing with deep inspiration. In outpatients, supervised/ observed spot specimen is collected on the first day of the visit, early morning sample on the following day at home and 3rd sample is collected when he visits the clinic on the 2nd day as observed spot. In clinics or wards, sputum samples should be taken in a designated place with good ventilation and sunlight, and away from other patients
Childhood Pulmonary TB	Sputum/ induced sputum/ BAL/ bronchial wash/ ET secretions/	Induced sputum best taken after fasting for 2-4 hours

Clinical syndrome	**Microbiology investigations**	**Special instructions**
	gastric lavage (if the child is unable to produce sputum) for - NAAT for MTB Mycobacterial culture	Gastric aspirate should be collected early morning, fasting and before mobilization Molecular assays (NAAT) should be used as the initial diagnostic test in children suspected of pulmonary and extra pulmonary TB
Adult and childhood Extrapulmonary TB		Send specimen in sterile, screw capped, transparent container without fixatives or preservatives such as formal saline
Musculoskeletal TB (including spinal TB)	Biopsy/ tissue/ pus/ aspirates for - NAAT for MTB Mycobacterial culture	For tissue/ biopsy: in prolong transportation, add 1mL sterile normal saline to prevent dehydration
Genito-Urinary TB	Urine for - Mycobacterial culture Collect three consecutive early morning, midstream urine samples, each sample 20-50mL Samples should be sent in separate culture bottles	Urine AFB is unreliable as it can be contaminated with atypical mycobacteria NAAT has low certainty of evidence for urine The external genitalia should be washed before specimen collection to minimize contamination. Urine should be collected on three consecutive days as the excretion of tubercle bacilli is intermittent
Central Nervous System TB including abscesses	Tissue/ CSF/ aspirates/ pus for NAAT for MTB Mycobacterial culture	Microscopy AFB on CSF has poor sensitivity NAAT should be used as a first line test when CNS TB with meningeal involvement is suspected Minimum 0.8mL of CSF should be sent for NAAT
Gastro intestinal TB	Tissue biopsy/ peritoneal and gastric fluid/ pus/ aspirates for - NAAT for MTB Mycobacterial culture	Histopathology will be useful

Clinical syndrome	**Microbiology investigations**	**Special instructions**
Pleural TB (including empyema)	Pleural fluid/ pleural biopsy tissue/ pus for - Plural fluid full report Mycobacterial culture NAAT for MTB	GeneXpert/ MTB of pleural fluid is not routinely recommended A pleural biopsy is preferable to pleural fluid as tubercle bacilli is mainly in the pleura Based on the clinical situation, the decision on pleural biopsy will be taken by the treating physician
Disseminated TB (including miliary TB)	Relevant samples are obtained after assessing patient by physical examination and imaging studies: lower respiratory tract specimens/ CSF/ pus/ urine/ aspirated fluid/ biopsy tissue/ bone marrow for - Mycobacterial culture NAAT for MTB	Blood samples are not tested by NAAT for MTB For mycobacterial culture and molecular studies, specimen should be collected into sterile screw capped container Transport as early as possible. If delay, store at +2 to 8ºC
Tuberculous lymphadenitis	Lymph node biopsy/ aspirates for - NAAT for MTB Mycobacterial culture	
Latent TB	Tuberculin skin test (Mantoux test) Whole blood in special tubes for - Interferon Gamma Release Assay (IGRA)	Performed in certain hospitals and chest clinics Performed only in special patient populations and in designated institutions Contact microbiologist for further information

Mycobacterial diagnostic services are available free of charge within the country in the following laboratories

a. NAAT assay sites

- National Tuberculosis Reference Laboratory (NTRL), Welisera (Telephone no. 0112 960 509)

- Chest Clinics - TH Anuradhapura, PGH Badulla, TH Batticaloa, TH Karapitiya, CC Kandy, GH Kurunegala, NHSL, PGH Ratnapura, GH Kegalle, NIHS, TH Jaffna, LRH, CC Polonnaruwa, GH Nuwara Eliya, GH Hambantota, AMH, CC Colombo, GH Vavuniya, GH Ampara, CC Puttalam, GH Trincomalee, GH Mannar, GH Matale, GH Matara, GH Monaragala, CSTH, Prison Hospital, Centre for TB Diagnosis & Education, Faculty of Medicine, University of Colombo

b. TB solid culture (LJ and Middlebrook 7H10 media) test sites

- National Tuberculosis Reference Laboratory (NTRL), Welisera
- Intermediate TB laboratories (ITLs) - Kandy/ Ratnapura/ Galle/ Jaffna
- Centre for TB Diagnosis & Education, Faculty of Medicine, University of Colombo

c. Automated liquid TB culture (MGIT) test sites

- National Tuberculosis Reference Laboratory (NTRL), Welisera
- Centre for TB Diagnosis & Education, Faculty of Medicine, University of Colombo

d. Interferon gamma releasing assays (IGRA) for latent TB diagnostic sites

- National Tuberculosis Reference Laboratory (NTRL), Welisera
- Centre for TB Diagnosis & Education, Faculty of Medicine, University of Colombo

References

1. Laboratory Manual for Tuberculosis Control - 5th edition, 2021
2. National Manual for Tuberculosis Control - 2021 update

CHAPTER 18

Urinary Tract Infections

Note:

- ❖ Refer Chapter 19 on Specimen Collection and Transport for further information
- ❖ Specify the method of urine collection such as midstream urine, urine obtained directly from renal, bladder or ureter during surgical procedures etc. as laboratory interpretation depend on the specimen type
- ❖ Do not send urine cultures in asymptomatic patients unless prior to urological procedure, pregnancy and early renal transplant

Clinical syndrome	Microbiological investigations	Special instructions
Acute uncomplicated cystitis	Urine full report Dipstick urine analysis for leukocyte esterase and nitrite Midstream urine in a sterile container for urine culture	Pus cells and RBCs could be present in other conditions as well Since antibiotic resistance is high in Sri Lanka, urine culture is recommended Test of cure (at the end of treatment) is not recommended If risk of STIs and/ or symptoms of urethritis is present, refer to the nearest STD clinic
Hemorrhagic cystitis	Urine culture Urine for - BKV, adenovirus PCR Day time urine sample for - Microscopy for *Schistosoma* ova	Consider non-infectious causes Collect urine into a sterile screw capped plastic containers and transport at +2-8ºC In immunocompromised patients In travellers from endemic countries
Acute pyelonephritis **Urosepsis**	Blood culture Midstream urine/ clean catch urine (in pediatric patients)/ catheter specimens/ suprapubic aspirate for - Urine culture	Typical symptoms of UTI may not present in urosepsis The method of urine collection should be mentioned in the request form for the laboratory to interpret the test results

Clinical syndrome	Microbiology investigations	Special instructions
Catheter-associated UTI	Urine collected from an indwelling catheter tube for - Urine culture	Routine urine culture testing of catheterized patients without systemic symptoms is not recommended Positive cultures should be interpreted carefully due to possible catheter colonization Do not collect the specimen from the catheter bag or do not send catheter tips for culture If a significant growth of *Candida* species is isolated, change the catheter and collect a fresh urine sample within 20 min of catheter insertion
Pyonephrosis/ obstructive uropathy **Perinephric abscess/renal abscess**	Blood culture Urine collected during stenting or PCN insertion for - Urine culture Radiologically guided pus aspirates/ pus collected during PCN insertion for - Bacterial culture	The method of specimen collection (e.g. urine obtained during stenting/ surgery) should be mentioned in the request form
Acute prostatitis **Chronic prostatitis**	Urine full report Midstream urine for culture Blood culture Midstream urine for - Bacterial culture Urine and expressed prostatic secretions (EPS) for - Microscopy wet mount Urine Modified Meares-Stamey 2-glass Test	Prostatic massage should not be performed in patients with acute prostatitis Seminal fluid culture is considered, if patient is not responding to treatment

Clinical syndrome	**Microbiologyinvestigations**	**Special instructions**
Suspected Renal tuberculosis (Sterile pyuria with no other cause)	First void midstream urine for three consecutive days (each sample 20-50mL) for - Mycobacterial culture Pus/ aspirated fluid for - NAAT for MTB Mycobacterial culture	Refer Chapter 17 on TB Direct smear urine for AFB is unreliable as urine can be contaminated with atypical mycobacteria

CHAPTER 19

Specimen Collection and Transport

A. Bacteriology

- Specimens should always be considered as infectious. Ensure adherence to strict safety measures by all healthcare workers from specimen collection, transport until handing over of the specimen to the laboratory.
- Label all the samples correctly, with matching information with the request form
- Include specific anatomical site and the side of the collected sample, in relevant specimen types such as wound, pus, eye etc.
- Ideally specimen collection should be done before starting antibiotics. However, initiation of antibiotics should not be delayed in acutely ill patients.
- The quantity of specimen collected must be sufficient to carry out all requested tests appropriately. Specimen for bacterial culture could be used for microscopy Gram stain.
- Do not refrigerate specimens for culture when suspecting *S. pneumoniae*, *H. influenzae, N. gonorrhea* as they are not viable at low temperature.
- Specimens should be transported in appropriate containers (specimen carriers, cool box) maintaining up-right position to prevent spillage and breakage while transport.For high consequence pathogens (infectious agents in Category A, UN 2814), specimens should be sent in triple package. Do not send the request form inside the package.

  Basic triple packaging: Primary receptacle with the specimen is water-tight and leak-proof or sift-proof receptacle wrapped with absorbent material to enclose and protect the primary receptacle. Secondary packaging is a leak-proof, watertight sealable container (usually plastic) and the third layer or the outer layer of packaging (protective packaging) that is used to protect the secondary packaging from physical damage while in transit. Usually use thick cardboard or plastic container as the outer most package.
- Send a duly filled, clean, legible request form with the specimen. Public sector hospital laboratories could use H1222 form. For testing at MRI, use the special request forms; Bacteriology/ MRI, Virology/ MRI, General form MRI
- Fill the request form completely with relevant socio-demographic and clinical details of the patient; brief clinical history, suspected diagnosis, relevant exposures to the organism, antibiotic treatment are critical information for the laboratory specialists to decide on the test method, period of incubation, interpret special and reference test results etc. to generate a reliable report.
- All samples should be sent to the laboratory immediately or within 2 hours of collection. If delay is unavoidable, specimens for bacterial cultures, should be stored and transported at room temperature and for antigen detection and molecular studies store and transport at +4 to 8ºC.

i. Clinical Microbiology

Diagnostic test	Collection Method	Special instructions
Blood culture for aerobic bacteria Automated blood culture bottle Conventional blood culture bottle	Volume of blood: Adults: 8-10mL (conventional bottles ~5mL, depending on the bottle size) Children: 3-5mL, Neonates: 1mL Follow manufacturer's instructions for automated bottles. *Preparation of the blood culture bottle:* o Select appropriate bottle; adult/ paediatric o Prior to use, examine the bottle for evidence of damage, deterioration or contamination. o Do not use bottles which exhibit visually evident signs; broth turbidity, pellicle formation, colour change, excess gas pressure o Check the expiry date before use o Label the bottle with matching information with request form (name, ward, BHT number, date and time) o Keep the culture bottle on a flat surface until it reaches room temperature o Disinfect the rubber stopper of the culture bottle with 70% isopropyl alcohol. *Patient site preparation:* o Palpate vein before disinfection of venipuncture site. o Clean the site concentrically with iodine followed by 70% alcohol and allow to dry. o Disinfect the rubber stopper of the culture bottle with 70% isopropyl alcohol. o Wear sterile gloves and draw blood. o Inject slowly into the bottle by piercing through the rubber stopper. o Mix the blood well with the broth by rolling the bottle in between palms immediately. **Automated blood culture bottle** Fill the automated adult blood culture bottles, until it reaches the level marked in the bottle. Do not cover the rubber stopper with plaster. Do not write, damage or disturb the 'Barcode label' on the bottle. Keep one on the bottle and remove the other 'Barcode sticker' and paste on request form.	Transport the bottle at room temperature as early as possible Do not refrigerate or incubate until it reaches the laboratory For *Brucella*, use an automated blood culture bottle General reporting procedure: First report is issued within 24-48 hours of incubation Thereafter, reports are issued only for positive blood cultures with the antibiotic susceptibility test results

Diagnostic test	Collection Method	Special instructions
	Conventional blood culture bottles prepared by microbiology laboratory Inoculate blood in to the bottle by piercing through the rubber hole or removing the lid of the bottle aseptically as appropriate.	
Central line blood culture	Collect blood from both central line and peripheral venous blood simultaneously and similar volume for suspected catheter-associated blood stream infection Clean the hub of the central line catheter with alcohol before collection of blood	Transport as soon as possible in RT Do not refrigerate
Intravenous catheter tip for culture central, arterial, Hickman, Broviac, peripheral, umbilical	Using a sterile scissor, cut the catheter tip in to 5 cm long segment from the distal end. Send in sterile, screw capped container with a lid Should collect blood from a peripheral site simultaneously	Send both bottles in RT to the laboratory as soon as possible.
Tissue for bacterial culture	Place the tissue collected during surgery into a sterile screw capped container. Add few drops of sterile saline to keep the small pieces of tissue moist.	Always submit asmuch tissue as possible. Do not submit swabs that has been rubbed over the surface of a tissue.
Tissue for molecular studies	Should collect into a sterile screw capped container with sterile normal saline, aseptically during surgery	Store and transport in cool temperature +4 to 8^{o}C.
Specimens for antigen detection tests	Blood/ CSF/ urine Collect into a sterile plain container	Store and transport in cool temperature +4 to 8^{o}C.

Diagnostic test	Collection Method	Special instructions
Urine culture	Clean catch or midstream urine (MSU) in adults; First voided morning sample is preferred. Collect midstream sample into a sterile, wide-mouth, screw capped container to fill 1/2 to 2/3rd of the container. Best before starting antibiotics. <u>Collection methods</u> Adult male and female patients: Patient should wash hands with soap and water before collection. Avoid contaminating the bottle mouth and inside the lid with hand while collecting the specimen. Females: External genitalia should be cleaned with soap and water. While holding the labia apart, begin to pass urine. After passing few mL, collect the MSU without stopping the flow of urine. Males: Clean the penis, retract the foreskin (if uncircumcised), and wash the glans with soapy water. While holding the foreskin retracted, begin voiding. After passing few mL, collect the MSU without stopping the flow of urine. Infants and young children, clean catch or midstream urine: The mother is instructed to clean the external genitalia of the child and to collect urine while taking precautions to avoid contamination. Catheter specimen: **No sampling port** Regarding the procedure of urine collection, read the manufacturer's instructions or contact urologist. Should obtain urine aseptically with a sterile needle and a syringe. Clean the tube with 70% alcohol. **With sampling port** Disinfect the catheter collection port with 70% alcohol. Stabilize the tube by holding it below the level of the sampling port. Insert the sterile syringe tip into the port. Aspirate minimum 10mL of urine.	Dispatch urine specimen to the laboratory at RT within 2 hours or if delay is unavoidable store and transport at +4 to 8^{o}C within 24 hours. Do not submit urine from bedpans or urinals for culture Do not submit 24-hour urine collection for culture Mention the urine collection method in the request form; MSU/ catheterized/ clean catch / surgical urine etc. for the laboratory interpretation of the test result Do not collect catheter urine unless patient is symptomatic Do not submit foley catheter tip, urine from catheter bags for culture Collect urine into a sterile, dry, screw capped, wide-mouthed container Transport to the laboratory within 2 hours

Diagnostic test	Collection Method	Special instructions
Cerebrospinal Fluid (CSF) culture (lumbar puncture, EVD, ventricular-peritoneal shunt)	Collect 0.5-1mL of CSF in to a sterile plain screw capped container before antibiotic treatment or before changing to a new antibiotic. CSF specimen collected from lumbar puncture: Collect CSF aseptically into a sterile screw capped container. For microbiology and molecular biology, send the 2nd or 3rd CSF specimen as soon as possible to the laboratory in RT. CSF from EVD/ Shunt; Clean the puncture site with 70% alcohol and aspirate using a sterile needle and syringe.	Initiation of antibiotics should not be delayed Obtain a blood for culture also from the patient Do not refrigerate the specimen as pneumococcus and *H. influenzae* are not viable at low temperatures Send the specimen to the laboratory immediately at room temperature
Sputum culture	Early morning, first expectorated sputum specimen is preferred. Collect ≥1mL into a sterile wide-mouthed screw capped container. Expectorated sputum: Ask the patient to gargle throat and rinse mouth with water. Avoid antiseptic mouth washes 8hrs before the procedure. Collect sputum by deep coughing into a sterile container. If expectoration is poor, postural drainage with the help of a physiotherapist may provide a better representative sample of lower respiratory tract.	Do not send salivary samples Do not refrigerate as pneumococcus and *Haemophilus* spp. are not viable at low temperatures Dispatch the specimen to the laboratory in RT immediately within 2 hours or at least less than 24 hours after collection For suspected tuberculosis, specimen collection is described in Chapter 17
Endotracheal (ET) secretions **Broncho-alveolar lavage (BAL)**	Collect 1-2mL of aspirated secretions from the ET tube with a suction catheter aseptically. BAL sample should be collected into a screw capped plain, sterile container during bronchoscopy	Transport without delay. A sample collected during chest physiotherapy provides a better representative sample of the lower respiratory tract

Diagnostic test	Collection Method	Special instructions
Throat swab for culture	Ask the patient to extend the neck. Depress the tongue with a tongue depressor to directly visualize the throat. With a sterile swab rub over tonsils soft palate, posterior pharynx and inflamed areas. Do not touch the tongue. Transport the specimen at room temperature.	Throat swab cultures are contraindicated in patients with epiglottitis.
Ear swab/ fluid aspirated during myringotomy for culture	Swabbing or aspiration should be done by a trained person. Pus or aspirate should be collected into a sterile screw capped container.	Transport to the laboratory as soon as possible at RT
Conjunctival swab for culture	Attending clinician should collect specimens. Sample each eye with separate swabs pre-moistened with sterile saline. Bed side inoculation is preferable as the amount of material is scanty. Inoculate the culture media first and then make a smear on a glass slide for Gram stain.	Sample both conjunctivae even if only one is infected, to determine the endogenous microflora. This can be used to compare the significance of the organism/s isolated from the infected eye. The specimen should be transported to the laboratory as soon as possible at room temperature
Corneal scraping for culture	Specimens collected by an ophthalmologist. Scrapings are collected after instillation of anesthetic drops. If conjunctival swabs are collected, specimens should be collected before the instillation of anesthetic drops which may inhibit some bacteria.	Fungal culture should be considered
Pus /aspirate for culture	Collect 1mL pus or aspirate ideally before starting antibiotics into a plain, sterile screw-capped container. Collect aspirate from the deepest part of the lesion.	Store at room temperature. Pus is a better specimen than pus swab for culture. Transport to the laboratory as soon as possible.
Wound swab for culture	Remove surface exudate by cleaning the wound with sterile normal saline to minimize the colonizing flora over the wound. Sample the base of the lesion or abscess wall in open wounds. Swab the deepest and most infected area of the wound with two swabs and put in to one container.	Store and transport as early as possible at room temperature. Tissue, pus or fluid is always superior to a swab. Dry swab may not yield growth.

Diagnostic test	Collection Method	Special instructions
Peritoneal / pericardial/ synovial fluid culture	Should be collected by trained medical staff. Collect 2-5mL fluid into a sterile plain screw capped container.	Specimens for culture should be transported immediately at RT
Anaerobic bacterial cultures	Collect at least 1mL fluid. Pus or tissue specimens should be collected into an anaerobic transport media (cooked meat broth) or into anaerobic blood culture bottles. Aspirated pus could be sent in a sealed syringe.	Store at room temperature and transport as soon as possible
MRSA screening	Use sterile swabs premoistened with sterile normal saline. Collect samples from nasal, axillae, groin and wound (if present) Swab both anterior nares with a single swab in a circular motion. Swab both axillae with a single swab rotating and brushing across the area. Similarly swab both groins.	Send in room temperature or if delay, refrigerate for < 24 hours.
Stool for culture	Collect diarrhoeal stool into a clean, wide-mouthed, disposable container preferably with a spoon. Fill until 1/3rd of the container. Ask the patient to defecate into a clean, dry, disinfectant-free bedpan. Stools should not be mixed with urine. Ideally, pus, blood, and mucous parts should be collected.	For the detection of diarrhoeagenic *E. coli, V. cholerae, Campylobacter* send specimens to Enteric Reference laboratory, MRI Send to the laboratory within two hours. If the delay is >2 hours, refrigerate (+4 to 8ºC) the specimen
Faecal swab for culture	Dip the swab into the stool from areas that appear bloody, slimy or watery. The quantity of specimen is important; visible fecal material must be present.	Store and transport at room temperature within 2 hours or use a special transport medium obtained from the laboratory.
Rectal swab for culture	Use a sterile cotton wool swab moistened with sterile saline or transport media. Insert the swab through the anal sphincter, rotate, and leave for 10 seconds and send in a sterile container	Send to the laboratory within 2 hours. If delay, use a transport medium obtained from the laboratory
Blood for serology	Collect blood, 3-5mL into a plain, sterile tube and allow to sit for 30min at room temperature for clot formation. Send the clotted blood specimen as it is or separate the serum aseptically in to a new sterile tube. Serum separation should not be done in wards. Transport within one day to the laboratory. If delay is unavoidable, store and transport at +2 to 8ºC in a cool box.	

ii. Molecular Biology - Bacteriology

- Specimens should be sent to the National Reference Laboratory for Clinical Microbiology & Molecular Biology (Room no. 336), Department of Bacteriology, Medical Research Institute, Colombo
- Report delivery is available by E mail, telephone and fax. Include relevant information in the request form to minimize the turn-around-time.
- The specimens for molecular biology should be transported at +2 to 8^{o}C in a cool box

Diagnostic test	**Specimen type**	**Best time of collection**
Detection of *Bordetella pertussis* and *B. parapertussis**	Nasopharyngeal aspirate (NPA)/ NP swab/ throat swab Swab by Dacron, rayon or nylon material is preferred	Within the first 3 weeks of illness
Detection of *Streptococcus pneumoniae**	Blood/serum/ CSF/ sterile fluids	Early illness within the 1st week
Detection of *Neisseria meningitidis**	Blood/serum/ CSF/ sterile fluids	Early illness within the 1st week
Detection of *Haemophilus influenzae**	Blood/serum/ CSF/ sterile fluids	Early illness within the 1st week
Detection of *Brucella* spp.**	Blood/ serum/ CSF	Early illness within 2 weeks
Detection of pathogenic *Leptospira* spp.**	Blood/ serum/ CSF/ fresh urine within 48 hours to reach the lab	Blood within the 1st week has a higher sensitivity CSF within 10 days Freshly-voided urine within the 1st week of illness
Detection of *Legionella pneumophila***	Respiratory specimens/ other clinical specimens	Specimens should be obtained only from clinically symptomatic patients

Method of detection:
* Bacterial Multiplex Realtime PCR
**Bacterial Singleplex Realtime PCR

iii. Microbiological investigations for the diagnosis of leptospirosis

- ❖ Investigations for confirmation of leptospirosis are performed at the National Reference Laboratory for Leptospirosis (NRLL Room No. 344), Department of Bacteriology, Medical Research Institute
- ❖ Fill the special request form with relevant clinical details of the patient, in addition to socio demographic information; brief clinical history, relevant exposures are important for the interpretation of special and reference test results for a reliable report
- ❖ Report delivery available by E mail, phone and fax. Include the relevant information for report delivery in the request form

Diagnostic Test	**Specimen type**	**Best time of collection**	**Transport condition**	**Remarks**
Molecular detection of pathogenic *Leptospira*	Blood 3mL Serum 2mL CSF 0.5-1mL Freshly-voided urine 10mL	Best within 7 days of illness Within 10 days of illness 1st week of illness	Collect into a plain sterile bottle Transport at +2 to 8°C in a cool box Within 48 hours to reach the lab	A confirmatory test. In early illness is preferred as the sensitivity is low after 7 days of illness Testing multiple urine samples in the first week is preferred
Microscopic Agglutination Test (MAT) by live pathogenic panel	Blood 5mL Serum 3mL Collect into a plain sterile container	1st sample: after 3-5 days of illness 2nd sample: 10-14 days apart	Transport at RT or if any delay, store and transport at +2 to 8°C in a cool box	A confirmatory test Serological reference method Negative result in early illness does not exclude leptospirosis
Blood culture for *Leptospira* Obtain special culture bottles fromNRLL, MRI Room no. 344	Two drops of blood into the special culture media bottle and mix immediately The broth should turn to cherry pink colour (not red)	Within 7 days of onset of illness Best before antibiotics	At room temperature, in dark cool place without direct sunlight	A confirmatory test. Important for identification of the reservoir host and for antibiotic susceptibility testing
Serology tests for screening	Available in few laboratories, <u>not at the NRL/MRI.</u> They are used as screening tests for probable diagnosis. The results of these tests should be confirmed by MAT. These kits should be validated locally before use as *Leptospira* serovar/strains show geographical variations			
Leptospira IgM detection by ELISA	Collect blood after the 5th day of illness Collect 3-5mL of blood or 3mL serum in a sterile plain tube Store at RT or if delay store and transport at +2 to 8°C in a cool box			
Leptospira rapid antibody test				

CHAPTER 19
Specimen Collection and Transport

B. Mycology

- For dry tissue specimens, add a small amount of sterile saline to keep the specimen moist
- All specimens should reach the laboratory within 72 hours of collection.
- All specimens should be sent at room temperature except for urine specimens. Urine samples must be processed as soon as possible. A delay longer than 2 hours at room temperature may impede the detection of some fungi. Therefore, should be stored at +4℃ if short delays are anticipated.

i. Medical Mycology

Mycology Investigation	**Specimen type & Method of collection**	**Remarks**
India ink stain Fungal culture Cryptococcal antigen test Direct microscopy and fungal culture	**CSF for CNS infection** Collect CSF 0.5-1mL in to a sterile container without a rubber lid **Blood for Cryptococcal antigen testing** See in specific serological tests in Mycology below **Tissue from brain biopsy** Place the tissue in a sterile screw capped container with sterile saline to keep the specimen moist	
Fungal culture	**Blood for invasive fungal infections** Collect 20mL of venous blood (0.5-1mL in neonates) into an automated blood culture bottle. Biphasic conventional blood culture bottles or conventional BHI bottles also can be used.	Specimen collection containers could be obtained from mycology/ microbiology laboratory
Fungal culture	**Bone marrow for invasive fungal infection** 2-3mL bone marrow aspirate into a sterile container with 0.5mL of 1:1000 sterile heparin Alternatively, 2-3mL of bone marrow aspirates collected into a paediatric automated blood culture bottle or into conventional BHI bottle	Specimen collection containers could be obtained from mycology/ microbiology laboratory
Direct microscopy and fungal culture	***Invasive fungal rhino-sinusitis*** **Sinus aspirates** Aspirates or tissue collected during FESS or surgery into a sterile screw capped container with sterile saline Tissue specimen from suspicious lesions into a sterile screw capped container with sterile saline	Histopathology is useful

Mycology Investigation	**Specimen type & Method of collection**	**Remarks**
Direct microscopy Fungal culture	**Sterile fluids** Aspirated ascites or paracentesis fluid from abdominal cavity, a representative portion of pleural, synovial and thoracentesis fluid. Obtain 5-10mL fluid and send in a sterile screw capped container	
Direct microscopy and fungal culture	***Fungal otitis externa*** **Specimens from external ear** Scrapings: use wooden or plastic spatula and send in sterile screw capped container Swabs: moist the swab with sterile saline or distilled water before obtaining the specimen and return the swab into the same sterile container	Scrapings are preferable than swabs
Direct microscopy and fungal culture	***Urinary tract infection*** **Urine** Early morning, clean catch urine/ urine obtained from a catheter one inch below the bifurcation/ suprapubic aspirate. Send in sterile screw capped container. If delayed more than two hours, should be stored and transported at +4℃	Urine from a catheter bag should not be sent *Candida* spp. may colonize or contaminate the urinary tract or catheters. Therefore, culture results should be interpreted carefully especially in catheterized and female patients
Direct microscopy and fungal culture	***Genital infections*** High vaginal specimens may be collected on swabs for recalcitrant yeast infections during speculum examinations Moisten the swab with sterile saline/ distilled water before obtaining the specimen. Swab should be sent in the same container with minimal delay.	

Mycology Investigation	**Specimen type & Method of collection**	**Remarks**
Direct microscopy and fungal culture	***Fungal keratitis*** **Ocular specimens** Collect purulent material from conjunctiva to two swabs and return to the same sterile container. Send without delay Corneoscleral donor rims/ corneal buttons/ corneal biopsies: should be sent in a sterile screw capped container with 1-5mL sterile normal saline Aspirated vitreous fluid: send in the same syringe with secured plunger with intact needle and cap Corneal scrapings: inoculate into culture media at the bed side and the remaining part should be placed on a slide. The slide smear should be covered with a cover slip. Transport immediately to the laboratory at room temperature.	Collect culture media (Sabouraud Dextrose Agar) and slides from the Mycology / Microbiology laboratory prior to the procedure
Direct microscopy and fungal culture	**Tissue or pus from abscess (subcutaneous / deep)** Remove surface exudate and clean with sterile saline or 70% ethyl alcohol. Send aseptically aspirated pus in sterile screw capped container Send surgically excised tissue in a sterile screwcapped container with sterile saline	Swabs are unacceptable
Direct microscopy and fungal culture Toluidine bluestain for PJP *Aspergillus* galactomannan antigen	**Respiratory specimens** Good quality sputum/ tracheal secretions/ BAL fluid in a sterile screw capped container Lung biopsy in a sterile screw capped container with few drops of sterile saline Good quality sputum/ induced sputum/ BAL fluid in a sterile screw capped container BAL/ bronchial wash (≥1mL) in sterile screw capped container/ whole blood (≥3mL) in **new** sterile plain tube. Should not sent in containers with rubber lids.	Do not send for Galactomannan in reused and re-sterilized containers, which may lead to false positive results Other respiratory specimens are not validated for the test

Mycology Investigation	**Specimen type & Method of collection**	**Remarks**
Direct microscopy and fungal culture	**Oropharyngeal candidiasis** Biopsies from oral lesions or eschars into a sterile screw capped container with sterile saline Saline rinse: Advise the patient to wash mouth and spit out and then rinse with 10 ml sterile saline for about 30 seconds and then spit into a sterile wide mouthed screw capped container Oral swab: Collect two oral swabs. Moisten the swab with sterile saline/ distilled water before collecting the specimen and return the swabs into the same sterile container	Saline rinses are preferable than swabs
Direct microscopy and fungal culture	**Skin biopsy** Send skin biopsies in a sterile screw capped container with sterile saline to keep it moist	Histopathology is useful
Direct microscopy and fungal culture	**Hair and scalp** Collect plucked hair roots, contents of follicles, skin scales from scalps. Cut hairs without roots are unacceptable. Wood's light can be used to select infected scalp hair. Send in clean folded paper (10x10cm)	
Direct microscopy and fungal culture	**Nail** Specimens should be collected from discolored, dystrophic, or brittle parts of the nail. Cut specimen as far back as possible and should include the full thickness of the nail. Remove any nail varnish, clean the nail with 70% alcohol. Scrape the superficial surface of the nail and discard the scrapings. Collect deep area scrapings, debris from under the nail. Send in clean folded paper (10 x 10 cm)	

Mycology Investigation	**Specimen type & Method of collection**	**Remarks**
Direct microscopy and fungal culture	**Skin scrapings** Clean the surface with 70% alcohol. Gently scrape outward from the active margin of the lesion with a scalpel to remove the superficial material and discard. Collect subsurface scrapings, top portion of vesicles (if present). Send in clean folded black paper (10 x 10cm) If minimal scaling is present, use a clear adhesive tape pressed on the lesion to remove material for examination. Place the adhesive side of the tape face down on a clean glass slide.	
Unacceptable specimens: Gastric contents/ gastric washings/ stool specimen/ urinary catheter tip		

ii. Fungal serological assays

Please note the following when requesting testing for Aspergillus specific Galactomannan:
Fungi and fungal spores are ubiquitous in the environment.
Heat stable Galactomannan antigen may not be destroyed by autoclaving and contaminate the specimen and lead to false positive results.
Therefore, the following precautions are essential.

- Use new, sterile, sealable plain tubes to collect blood
- Do not take used tubes after autoclaving. Heat stable Galactomannan antigen may not be destroyed by autoclaving and contaminate the specimen
- Avoid exposure to air as much as possible and quickly seal the tube properly
- Keep the tube for about 30min in room temperature to allow clot formation.
- Sending whole blood is encouraged to minimize possible contamination during serum separation. (Serum separation will be done at the Serology laboratory, Department of Mycology under strict aseptic conditions)
- Samples should be sent to the laboratory for processing as soon as possible after collection ideally on the same day of collection.
- If the sample is sent on the same day (within 24 hours) it can be kept at room temperature.
- If it cannot be sent to the Mycology laboratory within 24 hours of collection, suggest torefrigerate the specimen (+2 to 8ºC) for up to 4 days to prevent haemolysis.
- Do not freeze whole blood samples.
- If the delay in transport is inevitable, suggest to separate serum adhering to maximum precautions to prevent contamination. (Inside a biosafety cabinet by trained personnel, usingnew sterile pipette tips, new sterile sealable tubes etc.)
- If more than one test is requested from the same patient, ensure to send adequate volume.
- Specimens for antigen tests need stringent precautions to prevent contamination

Specific serology tests in Mycology

Test	Specimen / Container	Remarks
Aspergillus galactomannan antigen(ELISA)	Whole blood (= 3mL) in **New** sterile plaintube BAL/ bronchial wash (= 1ml) (in sterile screw capped container)	Re-used tubes will be Rejected Glass bottles with rubber lid will be rejected
Aspergillus IgG (ELISA)	Whole blood (= 2mL) in a plain tube	
Aspergillus immunodiffusion (precipitin) test	Whole blood (= 2mL) in a plain tube	
Total IgE (ELISA)	Whole blood (= 2mL) in a plain tube	
Candida mannan antigen (ELISA)	Whole blood (= 2mL) in a plain tube	
Candida antimannan antibody (ELISA)	Whole blood (= 2mL) in a plain tube	
Candida immunodiffusion (precipitin) test	Whole blood (= 2mL) in a plain tube	
Cryptococcal antigen test	Whole blood (= 3mL) in a plain tube	
Histoplasma urinary antigen (ELISA)	Random urine (= 1mL) sample, clean catch, midstream urine (= 1mL) as for collecting for urine culture Send in sterile screw capped container (without preservatives)	Transport in ice, store at +2 to 8^{o}C for up to 72 hours
Immunodiffusion (precipitin) test for *Histoplasma, Blastomyces, Coccidiomyces, Paracoccidiomyces*	Whole blood (= 2mL) in plain tube	

CHAPTER 19

Specimen Collection and Transport

C. Parasitology

Test	Specimen type and collection method	Storage and transportation
Intestinal helminths/ Cestode/ Trematodes		
Direct wet smear (saline and iodine) Concentration techniques Kato-Katz thick smear Culture for strongyloidiasis	**Faeces** Collect half a teaspoon of stool in a dry, clean, wide mouthed, leak proof container. Instruct to defecate directly into the container or on to a clean paper/ bedpan without disinfectants. Transfer the stool to the container immediately. Ensure feces are not mixed with urine, water, dirt, or other materials. If the first sample is negative, examination of 3 or more samples collected on separate days is recommended	Transport to the laboratory at room temperature. The specimens should be examined within 6 hours of passage as hookworm eggs tend to disintegrate Indicate possible immunosuppression of the patient
Scotch tape or Sellotape slide test for enterobiasis	**Peri-anal swab** Fold a strip of transparent adhesive tape over the end of a glass slide with the sticky side out. Wearing disposable gloves, separate the patient's buttocks with one hand and press the taped end of the slide against the skin around the anus in several places. Fold the tape back so the sticky surface adheres to the glass slide. For best diagnostic accuracy, perform the swab in the morning upon waking, before the patient defecates or bathe. Repeat testing over 3 days is necessary before excluding the infection	Transport at room temperature sealed in an envelope or plastic cover. Handle with care as the material is infectious.
PCR for detection of intestinal parasites	**Faeces** Same as for collection for intestinal helminths for direct faecal smear. Do not collect samples in glass containers or preserve in formalin as it can inhibit the PCR.	Transport at RT If delays in transport or analysis, store at +4ºC or -20ºC.
Identification of worms/ segments passed from the GI tract, migrated from	**Whole worms/ worm segments (of Cestodes)** Collected on passing.	Transport to the laboratory at room temperature without delay

Test	Specimen type and collection method	Storage and transportation
anus, nose, mouth etc.	Place the specimen in sterile normal saline in a screw capped plastic container. Transport to reference lab without delay. Do not preserve in 10% formalin as it complicates identification and inhibits PCR.	Handle specimens with care, as some can be infectious to human
Intestinal Protozoans		
Direct wet smear (saline and iodine) Modified acid-fast stain (cryptosporidiosis, cystoisosporiasis, cyclosporiasis) Concentration techniques	**Faeces** Collection is the same as for intestinal helminths. If blood and mucus are present in the stool, collect the sample from the areas where they are observed. If a trichrome stain (permanent stain) is required, request a container with polyvinyl alcohol (PVA) preservative from the reference laboratory beforehand. The trophozoites of *Entamoeba* and *Giardia* spp. disintegrate within 30 minutes after passage. Informing the laboratory beforehand and immediate examination is mandatory.	Same as for intestinal helminths. Transport immediately (within 30 minutes of passage) at room temperature if trophozoites need to be identified.
Extra-intestinal amoebiasis (amoebic liver abscess)		
Direct wet smear of liver abscess aspirate PCR	**Liver abscess aspirate** Radiologically guided aspiration shouldbe performed by a trained medical officer, from the edge of cyst (wall)/ or the terminal part of the aspirate. The trophozoites of *Entamoeba histolytica* disintegrate within 30 minutes after passage, hence informing the laboratory beforehand and immediate examination is mandatory	**For microscopy,** Transport immediately (within 30 minutes) at room temperature. Specimens should be sent in a sterile plain bottle or in the syringe with the needle capped. Mention the time when the aspiration was performed. For PCR, refrigerate and transport in ice.

Diagnosis of blood parasites: Malaria		
Thick and thin blood smears Malaria rapid diagnostic test (RDT) Malaria PCR	**Blood** Collect 2-3mL of blood in an EDTA tube (one sample should be sent to AMC) Alternatively capillary/ finger-prick blood canbe used for microscopy. Blood samples should be collected as soon as malaria is suspected and ideally collected during a fever spike before starting treatment. **Collection of capillary blood** Follow safety precautions. Clean the middle/ ring finger of the non-dominant hand (big toe in children) with alcohol. Puncture the ball of the finger laterally with a sterile lancet, palm facing up. Wipe away the first drop with a cotton swab. Apply gentle pressure to collect a drop of blood in the center of the slide for the thin film. Collect 2-3 drops to make about a 1cm coin smear. If malaria is strongly suspected, but initial tests are negative, do at least three consecutive blood smears and RDTs before excluding it. If venous blood cannot be collected, collect three blood spots (125µL each) on filter paper for PCR analysis.	Blood should be stored and transported at +4ºC in a cool box. Smears on slides should be air dried thoroughly and packed in an insect-proof container. Transport at room temperature. Seal dried blood spots in a polyethylene cover, place them in an envelope, and transport at room temperature. All suspected patients must be informed to the Anti Malaria Campaign with a blood sample for reconfirmation.

Diagnosis of blood parasites: Filariasis		
Thick blood smear (Giemsa stained) for the detection of microfilaria Concentration of blood for detection of microfilaria Rapid diagnostic test for circulating filarial antigen (CFA) of *Wuchereria bancrofti* Antibody detection by ELISA	**Blood** Capillary/ finger-prick blood or venous blood collected between 10pm-2am should be used for the microscopic detection of microfilaria. **For antigen** detection, whole blood samples in EDTA can be collected at anytime of the day. **For antibody** detection, 2-3mL of venous blood should be collected in a plain tube. **Collection of capillary blood is as same as for malaria.** Prepare two, coin shaped smears of even thickness. Air dry. If smear preparation cannot be done immediately, collect venous blood in heparin/EDTA.	Blood/serum should be stored in a refrigerator (4ºC) and transported in a cold box (4ºC). Smears on slides should be air dried thoroughly and packed in an insect proof container and transported at room temperature.
Cutaneous leishmaniasis		
Giemsa-stained smear for detection of amastigotes Culture Polymerase chain reaction	For all procedures, follow safety precautions and sterile techniques. Clean the lesion with 70% alcohol. Do not use povidone iodine. **Slit-skin scrapings (microscopy)** Collect from the active edge of the lesion. Make a slit along the active edge of the lesion with a sterile no. 11 scalpel. Gently scrape tissue from both edges of the slit using the scalpel. Smear the scrapings on to a clean glass slide and dry. **Lesion aspirates (microscopy/ culture/ PCR)** Aspirate the active edge of the lesion or prominent nodule with 0.2 to 0.3 mL of sterile saline injected to the site, using 22/ 23 gauge sterile needle.	The samples could be transported at room temperature. The air-dried smears should be packed in an insect proof container and transported at room temperature. The aspirates/ biopsies for culture and PCR should be refrigerated (4ºC or -20ºC) and transported in ice if delay is expected. Check the availability of tests from the reference laboratory prior to collecting samples.

Cutaneous leishmaniasis ct.	Place one drop of the aspirate on a clean glass slide and make a smear using the needle. **Punch biopsies (PCR/ culture)** Take 2mm punch biopsies from the active edge of ulcers or prominent nodules following standard dermatological procedures. Place the biopsy in a sterile microcentrifuge tube (provided by the reference lab) with 1mL of sterile normal saline, **without formalin**, for PCR and culture. **Impression smears/ touch smears (microscopy)** Can be performed simultaneously when obtaining a biopsy. The tissue specimen after making an impression smear, will not be sterile but it remains useable for PCR. Hold the punch biopsy specimen with forceps and press it on a clean glass slide using a rolling or circular motion. Air dry.	
Visceral leishmaniasis		
Giemsa-stained smear for detection of amastigotes Culture PCR Rapid diagnostic test for detection of RK39 antibodies (screening)	**Bone marrow, whole blood in EDTA (for buffy coat) and lymph node, splenic or liver aspirates** **The tissue aspirates/ biopsy** should be performed by a qualified medical officer under strict aseptic conditions. **For microscopy:** Place few drops of aspirate on a clean, dry, labelled glass slide, make a smear and air dry. Multiple smears recommended. **For culture:** Collect the aspirate to a sterile, dry plain bottle or send in sterile normal saline. **For PCR:** Collect 2-3 mL of bone marrow aspirate to a sterile EDTA bottle or other specimens as for culture. **For RK39 (antibody detection):** Collect 2-3 mL of venous blood to a plain tube and allow to stand for 30 minutes for serum separation.	Slides with smears should be transported at room temperature, packed in an insect proof container. For other tests, if there is a delay in transport, send on ice Storage of specimens till DNA extraction can be at 4ºC or -20ºC. Transport serum on ice.

Trichomoniasis		
Direct microscopy(wet mount and/ or Giemsa-stained smear)	**Female**: Endocervical swab/ high vaginal swab from the posterior fornix **Male**: urethral swab/ first-void urine **High vaginal swab** Insert a saline-moistened speculum. Insert a dry swab into the posterior fornix to collect vaginal material. Press the swab against the vaginal wall and withdraw it. Place a large drop of saline on a microscope slide and emulsify the swab in the saline to make it turbid. Carefully add a coverslip without trapping air bubbles. **Urethral discharge** Collect the sample from the male urethra with a cotton tip applicator. Place a large drop of saline on a microscope slide and emulsify the swab in the saline to make it turbid. Carefully add a coverslip without trapping air bubbles.	Examine the specimen within 20 minutes of collection to prevent the parasite from becoming nonmotile and dying, which would make the diagnosis difficult. Examination is preferably performed as a bed side test If sending the sample to a lab, insert the applicator stick into a test tube with 0.5-1 mL of 0.9% saline and send without delay at room temperature Do not refrigerate samples
Toxoplasmosis		
IgM and IgG antibody detection byELISA PCR	**Blood/ serum /cerebrospinal fluid for serology and PCR** **Blood:** Collect blood into a plain tube for serology. For PCR, collect blood to an EDTA tube. For serology, paired samples should be tested 4-6 weeks apart to identify seroconversion or rising titre. For IgM antibodies, collect sample by the 5th day of infection. IgG antibodies, usually appear after the second week of infection. **Lumbar puncture:** Collect 0.5-1mL of CSF in a sterile, screw capped, plastic vial according to the standard protocol under strict aseptic conditions.	Blood for serology: Send to the laboratory preferably within two hours. If delayed, store in refrigerator and transport +2-8^{0}C in a cool box. Blood/ CSF for PCR: Transport +2-8ºC in a cool box to the laboratory. Store at +4ºC or -20ºC.

Trichomoniasis		
	Urine: Obtain 20-50mL of first void urine of the day void to a sterile, screw capped, plastic container. For urine, 20-50mL of first void urine of the day after centrifugation. If not, urine should be collected one hour after the previous void.	

Toxocariasis		
IgG antibody detection by ELISA	**Blood** Same as for toxoplasmosis.	Same as for toxoplasmosis.
Acanthamoeba infection (keratitis/ meningoencephalitis)		
Microscopy PCR	**Corneal scrapings or biopsy** **Cerebrospinal fluid** **Corneal scrapings:** Should be collected by a qualified medical officer under strict aseptic conditions into a sterile screw capped container with sterile saline. **Lumbar puncture:** Collect 0.5-1mL of CSF in a sterile, screw capped, plastic vial according to the usual protocol under strict aseptic conditions.	Transport immediately at room temperature. If delay anticipated, transport +2-8^{o}C in a cool box

***Naegleria sp.* infection** (suspected in meningoencephalitis in the immunocompetent)		
Microscopy PCR	**CSF** Same as for *Acanthamoeba sp.*	Same as for *Acanthamoeba sp.*
Dirofilariasis		
Dirofilaria spp worms Identification of worms extracted from skin nodules/sclera	**Whole/ parts of worms** Extracted from nodules/ sclera Place the specimen in sterile normal saline or 70% alcohol into a sterile, screw capped plastic container.	Transport to referencelab without delay. Do not place specimens in 10% formalin, because formalin causes the worm to contract and inhibits PCR.

CHAPTER 19

Specimen Collection and Transport

D. Virology

Specimen	Collection method	Storage and transportation
Blood for antigen detection	Collect 2-3mL of blood in to a sterile plain plastic tube. Collect blood in the first 5 days of illness. Collection procedure is same as for serology.	Store and transport at +4 to 8ºC If delay is >48h, serum should be separated and stored at +4 to 8ºC
Blood for serology	**Detection of IgM** A single sample of 2-3mL blood should be collected in to a sterile plain tube after day 5 of onset of illness **Detection of IgG** Paired blood samples (acute & convalescent) 7 -14 days apart are required to demonstratea four-fold antibody rise or seroconversion. The acute serum sample should be obtained as soon as possible during illness, no later than 5-7 days after onset of the illness.	If stored at +4 to 8ºC, this temperature should be maintained during transport
	Collection procedure 2-5mL of venous blood into a labelled, clean, sterile, dry, screw capped container (preferably plastic) Keep at room temperature for 30 minutes to 1 hour for the clot to form and retract. Indicate the date of onset of illness, date of collection in the request form.	
Specimens for molecular studies	Blood samples should be collected into a plastic tube with EDTA Other samples except blood, CSF and stools, should be collected into VTM	Store and transport at +4 to 8ºC Samples should be sent to the virology laboratory within 48 hours of collection
CSF	Collect 0.5-1mL into a sterile screw capped plastic container	Store and transport at +4 to 8ºC. Samples should be sent to the virology laboratory within 48hrs

Specimen	Collection method	Storage and transportation
Respiratory specimens	**Nasopharyngeal or nasal swab** Insert a flexible, fine-shafted swab into the post-nasal space. Rotate the swab and let swab rest in place for several seconds to absorb secretions. Use the same or separate swabs for each nostril Place both swabs in the tube with viral transport medium (VTM)	Store and transport at +4 to 8°C Sample should be sent to a virology laboratory within 48 hours of collection Swabs should be rayon or Dacron with plastic shaft
	Oropharyngeal or throat swabs Swab both tonsil areas and place the swab in VTM Use a tongue depressor to depress the tongue so that contamination of swab with saliva is prevented. Both nasal swabs and one throat swab from the same patient should be transported in one tube with VTM	
	Nasopharyngeal aspirate (NPA) Using a mucous collection device (disposable mucous extractor), insert an NG tube into the posterior nasopharynx. Apply suction, using intermittent suction as the catheter is withdrawn. Wash aspirate through tubing using 2mL of VTM	
	Broncho-alveolar lavage (BAL) and Tracheal aspirate Place 3-5mL in a sterile plastic container with or without VTM depending on the storage and transport time to reach the laboratory	
	Tracheal secretions collected with swabs Collected during surgical procedure or at the postmortem	
	Lung Biopsy (at post mortem) Obtain through intercostal space using a true cut biopsy needle as early as possible after death for virus isolation. Collect into viral collection tubes with 3-5mL VTM For PCR - Collect into VTM or sterile normal saline. Do not use formalin or alcohol.	
Vesicular fluid and Skin scrapings	Prior cleaning may inactivate the viruses Aspirate vesicular fluid with a fine needle attached to a 2mL syringe. Place the aspirated fluid in a sterile container with 2mLVTM. Swab the open lesions to obtain both fluid and cells from the base of the lesion.	Store and transport at +4 to 8°C Sample should be sent to a virology laboratory within 48 hours of collection

Specimen	Collection method	Storage and transportation
Ocular specimen	**Conjunctival swab** Swab the lower conjunctiva with a flexible, fine- shafted swab premoistened with saline Place swab in VTM aseptically **Corneal/conjunctival scrapings** Collected only by ophthalmologists or trained medical staff. Place in a sterile externally screw capped containers with VTM (e.g. cryovial)	Store and transport at +4 to 8ºC Sample should be sent to a virology laboratory within 48 hours of collection
Stool	**For Acute Flaccid Paralysis (AFP) surveillance** Collect 8-10 g of stools (size of 2 tamarind seeds) into a wide-mouthed, leak-proof, externally threaded, screw capped, clean, dry container Collect 2 stool samples at least 24 hours apart within 14 days of onset of paralysis. Collect samples from all age groups. Label the specimen with date and time of collection.	Store and transport at +4 to 8ºC Sample should be sent to a virology laboratory within 48 hours of collection
	For stool virus detection Collect single stool sample as for AFP as above during the acute stage of illness	
Rabies diagnosis Antemortem samples	**Pooled Saliva** Collect saliva from the oral cavity using a syringe or a dropper. Repeat the procedure in one-hour intervals for 3 to 4 times. Pool the collected saliva in to one container with VTM. Keep the container at +4ºC until transport. In situations where aspiration is not possible, repeatedly swab the oral mucosa thoroughly using 3-4 swabs at one-hour intervals and send in VTM at +4ºC. If VTM is not available saliva can be sent in sterile normal saline.	Transport the sample maintaining +4 to 8ºC to the Rabies reference laboratory with relevant clinical details of the patient; clinical features, present condition, and the exposure Contact the Department of Rabies, MRI for further information
	CSF CSF samples should be collected into sterile, dry containers without preservatives or VTM.	Store and transport at +4ºC.

Specimen	Collection method	Storage and transportation
Rabies diagnosis Postmortem specimen **Human brain**	Whole, fresh brain without preservatives should be transported. The specimen should be stored and transported at +4ºC in a leak proof container. Send the specimen with the request form with relevant clinical history at earliest possible	Should maintain the cold chain (+4ºC) until reach the laboratory
Rabies diagnosis Postmortem specimen **Animal head**	Brain tissue is the only postmortem sample for testing. Animal's head or if, in a case of a small animal the whole carcass should be sent to the laboratory as soon as possible. Head should be placed in leak proof polythene bag/container (primary container) Do not fix the head in formalin or do not submit live animals.	If transport delay is >8 hours following the death of animal, the head should be sent at +4 to 8ºC in a cool box Ice should be placed inside a box (secondary container) in between primary and secondary container. The head should not be directly in touch with ice

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