SRI VENKATESWARA INSTITUTE OF MEDICAL SCIENCES

(A University established by an act of A.P. State legislature)

TIRUMALA TIRUPATI DEVASTHANAMS, TIRUPATI - 517501

CLINICIAN'S REFERENCE MANUAL

DEPARTMENT OF MICROBIOLOGY

Scope: What is this manual about?

This manual is designed to provide an overview of the services offered by the department of Microbiology and serve as a quick reference guide for all users.

Laboratory Management is committed to ensure stringent adherence to quality in all laboratory procedures that meet requirements of internal and external quality assessment tests (EQAS by CMC Vellore) and in accordance with requirements of the ISO 15189

Document Control

Electronic version of this manual is available on SVIMS website. (Access at- www.svimstpt.ap.nic.in)

Location

The department of Microbiology located in Room No.20, SVIMS Padmavathi emergency & OPD building.

Contact Us

Postal Address:

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Getting Started

LOCATION OF THE DEPARTMENT: IInd floor of SVIMS Padmavathi emergency & OPD building:

Sections: Bacteriology, Serology, Immunology, Mycology, Mycobacteriology, Molecular Microbiology, Parasitology.

DEPARTMENTAL WORKING HOURS:

The Department is open 24x7 on 365 days of a year

DEPARTMENT FACULTY & CONTACT NUMBERS:

		CONTACT NUMBERS
SECTIONS/LABS	Office/PA	0877 - 2287777- 2243
	Lab Testing/Reports Enquiry (Reception)	0877 - 2287777- 2254
	Bacteriology	0877 - 2287777- 2260
	Mycobacteriology	0877 - 2287777- 2366
Faculty	Dr. B. Venkata Ramana HOD i/c	0877 - 2287777- 2495

FACULTY/SENIOR RESIDENTS PHONE NUMBERS

1	DR. B.VENKATARAMANA	ASSOC. PROF & HOD I/C	94943 65763
2	DR. ABHIJITH CHOUDHURY	PROFESSOR	94925 70422
3	DR. R.JAYAPRADA	ASSOC. PROF	94923 66555
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6	DR. S. YAMINI	ASST. PROF	81421 41178
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SERVICES OFFERED & CHARGES: DEPT. OF MICROBIOLOGY

	MICROBIOLOGY	CHARGES per TEST	
CODE	Investigation_Name	OP/OI	IP
MIC001	ACCP ELISA	750	925
MIC002	AFB (SMEAR)	90	110
MIC003	AFB (SMEAR)(AF)	90	110
MIC004	AFB (SMEAR)(CSF)	90	110
MIC005	AFB (SMEAR)(OTHER)	90	110
MIC006	AFB (SMEAR)(PF)	90	110
MIC007	AFB (SMEAR)(PUS)	90	110
MIC008	AFB (SMEAR)(SPUTUM)	90	110
MIC009	ANA ELISA	400	500
MIC010	ANA PROFILE	1300	1625
MIC011	ANTI CARDIOLIPIN ELISA	825	1030
MIC012	ANTI PANCA CANCA GBM	1250	1560
MIC013	ASCITIC FLUID CULTURE AND SENSITIVITY	325	400
MIC014	ASO TITRE	300	375
MIC015	BILE FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC016	BLOOD CULTURE	400	500
MIC017	BLOOD CULTURE BACT/ALERT	600	750
MIC019	BODY FLUID CULTURE AND SENSITIVITY	325	400
MIC020	BONE MARROW FOR CULTURE AND SENSITIVITY	325	400
MIC021	BRONCHIAL LAVAGE FOR CULTURE AND SENSITIVITY	325	400
MIC022	C3 C4	1400	1750
MIC023	CATHETER TIP FOR CULTURE AND SENSITIVITY	325	400
MIC024	CERVICAL SWAB FOR CULTURE AND SENSITIVITY	325	400
MIC026	CRP	250	325
MIC027	CSF CULTURE AND SENSITIVITY	325	400
MIC028	CSF INDIA INK PREPARATION	90	110
MIC029	CVP CATHETER TIP FOR CULTURE AND SENSITIVITY	350	450
MIC030	C V P TIP FOR CULTURE AND SENSITIVITY	350	450
MIC032	DISCHARGES CULTURE AND SENSITIVITY	325	400
MIC033	DRAIN FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC034	DRAIN TIP FOR CULTURE AND SENSITIVITY	325	400
MIC035	DS DNA ELISA	400	500
MIC036	EAR SWAB FOR CULTURE AND SENSITIVITY	325	400
MIC037	EXPRESSED PROSTATE SECRETION (EPS) CULTURE AND SENSITIVITY	600	750
MIC038	ENDOTRACHEAL (E.T) ASPIRATION FOR CULTURE AND SENSITIVITY	325	400
MIC039	E.T(ENDO TRACHEAL) TIP FOR CULTURE AND SENSITIVITY	350	450
MIC040	EXTERNAL VENTRICULAR DRAIN (E.V.D) TIP FOR CULTURE AND SENSITIVITY	350	450
MIC041	FEMORAL TIP FOR CULTURE AND SENSITIVITY	350	450
MIC042	FOLEYS CATHETR TIP FOR CULTURE AND SENSITIVITY	350	450
MIC043	FUNGAL CULTURE(CSF)	325	400

MIC044	FUNGAL CULTURE(OTHER)	325	400
MIC045	FUNGAL CULTURE(PUS)	325	400
MIC046	FUNGAL CULTURE(SPUTUM)	325	400
MIC047	FUNGAL CULTURE(URINE)	325	400
MIC048	FUNGAL (KOH) (OTHER)	90	110
MIC049	FUNGAL (KOH) (PUS)	90	110
MIC050	FUNGAL (KOH) (SPUTUM)	90	110
MIC051	FUNGAL (KOH) (URINE)	90	110
MIC052	GASTRIC ASPIRATION FOR CULTURE AND SENSITIVITY	325	400
MIC053	GRAMS STAIN(ASCITIC FLUID)	90	110
MIC054	GRAMS STAIN(CSF)	90	110
MIC055	GRAMS STAIN(ET)	90	110
MIC056	GRAMS STAIN(OTHER)	90	110
MIC057	GRAMS STAIN(P.D FLUID)	90	110
MIC058	GRAMS STAIN(PLEURAL FLUID)	90	110
MIC059	GRAMS STAIN(PUS)	90	110
MIC060	GRAMS STAIN(SPUTUM)	90	110
MIC061	GRAM STAIN (C V P TIP)	90	110
MIC063	HBSAG ELISA	300	375
MIC064	HBSAG RAPID TEST	300	375
MIC065	HCV ELISA	660	825
MIC066	HCV RAPID TEST	660	825
MIC067	HCV VIRAL LOAD	1600	2000
MIC068	HIV ELISA	300	375
MIC069	HIV RAPID TEST	300	375
MIC070	HIV VIRAL LOAD	1600	2000
MIC071	IMMUNOFLUORESCENCE ANA	650	800
MIC072	I.V CANNUALA TIP FOR CULTURE AND SENSITIVITY	350	450
MIC073	JUGULAR TIP FOR CULTURE AND SENSITIVITY	350	450
MIC074	LEPTOSPIRA ELISA	350	425
MIC076	MANTOUX	90	110
MIC077	MYCO BACTERIAL CULTURE(CSF)	530	660
MIC078	MYCO BACTERIAL CULTURE MGIT	500	630
MIC079	MYCO BACTERIAL CULTURE(OTHER)	500	630
MIC080	MYCO BACTERIAL CULTURE(PUS)	500	630
MIC081	MYCO BACTERIAL CULTURE(SPUTUM)	500	630
MIC082	MYCO BACTERIAL CULTURE(URINE)	500	630
MIC083	NASAL SWAB FOR CULTURE AND SENSITIVITY	325	400
MIC085	HSV REAL TIME PCR/QUANTITATIVE DNA DETECTION	1600	2000
MIC086	HBV REAL TIME PCR/QUANTITATIVE DNA DETECTION	1600	2000
MIC087	HCV REAL TIME PCR/QUANTITATIVE RNA DETECTION	1600	2000
MIC088	HIV REAL TIME PCR/QUANTITATIVE RNA DETECTION	1600	2000
MIC089	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTIONN (CSF)	1600	2000
MIC090	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTIONN (OTHER)	1600	2000
MIC091	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTION (PLEURAL FLUID)	1600	2000

MIC092	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTION (PUS)	1600	2000
MIC093	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTION (BODY FLUID)	1600	2000
MIC094	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTION (SPUTUM)	1600	2000
MIC095	MTB REAL TIME PCR/QUANTITATIVE DNA DETECTION (URINE)	1600	2000
MIC096	P.D FLUID (KOH)	90	110
MIC097	PERICARDIAL FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC098	PERITONIAL DIALYSIS FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC099	PERITONIAL FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC100	PLEURAL FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC101	PROCALCITONIN	1000	1250
MIC102	PROTEIN 'C ' ELISA	2300	2450
MIC103	PUS CULTURE AND SENSITIVITY	325	405
MIC104	RA FACTOR	200	250
MIC105	SCRUBTYPHUS ELISA	400	500
MIC106	SEMEN CULTURE AND SENSITIVITY	325	400
MIC107	SPUTUM CULTURE AND SENSITIVITY	325	400
MIC108	STOOL CULTURE AND SENSITIVIY	200	250
MIC109	STOOL FOR CRYPTOSPORIDIUM	90	110
MIC110	STOOL FOR MICROSCOPIC EXAMINATION	90	110
MIC111	STOOL HANGING DROP PREPARATION	90	110
MIC112	STOOL (OVA AND CYST)	90	110
MIC113	SUCTION TIP FOR CULTURE AND SENSITIVITY	350	450
MIC114	SYNOVIAL FLUID FOR CULTURE AND SENSITIVITY	325	400
MIC115	THROAT SWAB CULTURE AND SENSITIVITY	325	400
MIC116	TISSUE FOR CULTURE AND SENSITIVITY	350	450
MIC117	TREPONEMA PALLIDUM HEMAGGLUTINATION TEST (T.P.H.A)	325	400
MIC118	TRACHEAL SWAB FOR CULTURE AND SENSITIVITY	350	450
MIC119	TRACHEAL TIP FOR CULTURE AND SENSITIVITY	350	450
MIC120	URINE CULTURE AND SENSITIVITY	200	250
MIC122	VENEREAL DISEASE RESEARCH LABORATORY (VDRL) TEST	200	250
MIC123	VDRL(CSF)	200	250
MIC124	VEGINAL SWAB FOR CULTURE AND SENSITIVITY	325	400
MIC125	VENTRICULOPERITONEAL (V.P.) SHUNT TIP FOR CULTURE AND SENSITIVITY	350	450
MIC126	WATER CULTURE	250	310
MIC127	WESTERN BLOT HIV	1600	2000
MIC128	WIDAL TEST	200	250
MIC129	NEURONAL ANTIGENS PROFILE (IgG)	2500	3125
MIC130	DRUG SUSCEPTIBILITY TESTING FOR MYCOBACTERIUM TUBERCULOSIS	1500	1875
MIC131	ENDOMYSIUM IgA BY ELISA	700	875
MIC132	ANTI GBM IgG ELISA	1050	1250

MIC138	BRUCELLA BLOOD CULTUREM (BACT 'T' ALERT)	600	750
MIC139	ANAEROBIC CULTURE FOR ALL SPECIMENS	325	400
MIC141	MYCOBACTERIUM CULTURE & SENSITIVITY	1600	2000
MIC142	ANTI HBS AG ELISA	300	375
MIC144	ds DNA IgG ELISA	400	500
MIC145	HBs Antibodies ELISA	300	375
MIC146	OPERATION THEATRE SWABS TESTING	250	250
MIC147	ENVIRONMENTAL AIR SURVEILLANCE	250	250
MIC148	WATER SAMPLES	250	310
MIC149	BLOOD BAG STERILITY TESTING	1000	1000
MIC150	HD DIALYSATE SOLUTION	250	250
MIC151	PD DIALYSATE SOLUTION	250	250
MIC152	RO SOLUTION	250	250
MIC157	ANTI CARDIOLIPIN (ACL) IgG ELISA	825	1030
MIC158	ANTI CARDIOLIPIN (ACL) IgA ELISA	825	1030
MIC159	PROTEIN 'S' ELISA	2300	2450
MIC160	VDRL ELISA TEST	200	250
MIC161	VIRAL MARKERS (TRIDOT & ELISA) FOR HIV/HSV/HBV	1600	2000
MIC162	ENDOTOXIN END POINT CHROMOGENIC LAL ASSAY FOR WATER SAMPLES	2000	2500
MIC166	FAECAL CLOSTRIDIUM DIFFICILE TOXIN A & B ELISA	900	1100
MIC167	DISINFECTANT TESTING	250	310
MIC168	SERUM / CSF QUALITATIVE TEST FOR CRYPTOCOCCAL ANTIGEN LATEX AGGLUTINATION DETECTION	600	700
MIC169	SERUM / CSF QUANTITATIVE TEST FOR CRYPTOCOCCAL ANTIGEN LATEX AGGLUTINATION DETECTION	1000	1200

SERVICES TURN AROUND TIME:

	Test	TURN AROUND TIME (TAT)			
		Waiting time	**Time to Prepare the test**	**Time to Prepare and Release the report**	**Total TAT**
1.	Gram stains	1hr	1hr	1hr	3 hrs
2.	Stool for Ova & Cyst	1hr	1hr	1hr	3 hrs
3.	Aerobic Culture & Sensitivity (Pus , All body fluids, discharges, CSF, all type of Catheters etc.)	1hr	4days	1hr	4days
4.	Aerobic Culture & Sensitivity (Urine, sputum)	1hr	3 days	1hr	3 days
5.	Aerobic Blood Culture & Sensitivity	1hr	8 days	1hr	8 days
6.	AFB(Z.N) staining	1hr	1hr	1hr	3 hrs.
7.	Mycobacterial(AFB)Cultures/ Culture & Sensitivity (All Samples)	1hr	8 weeks	1hr	8 weeks
8.	MTB Diagnosis by GeneXpert	1hr	22hr	1hr	1 day
9.	KOH mount	1hr	2-4hr	1hr	4-6 hrs.
10.	India Ink preparation	1hr	1hr	1hr	3hrs
11.	Fungal Cultures (various Samples)	1hr	2days-4 weeks	1hr	2 days -4 weeks
12.	Procalcitonin (PCT)	1hr	30mins	30mins	2hrs
13.	HBsAg, HIV(RVabs) &HCV Rapid tests	1hr	1hr	1hr	3 hrs
14.	ASO TITRE	1hr	2hr	1hr	4 hrs
15.	CRP test	1hr	2hr	1hr	4 hrs
16.	R.A FACTOR	1hr	2hr	1hr	4 hrs
17.	VDRL test (Blood &CSF)	1hr	2hr	1hr	4 hrs
18.	WIDAL test (Quantitative)	1hr	22hr	1hr	1day
19.	TPHA	1hr	22hrs	1hr	1 day
20.	Neuronal Antigen profile-2	1hr	22hrs	1hr	1 day
21.	HBsAg , HIV(RVabs) & HCV by ELISA method	1hr	22hrs	1hr	1 day
22.	HIV (RV abs) Western blot Assay	1hr	1 day	1 hr	1day
23.	Mantoux test (PPD)	1hr	3 days	1hr	3 days
24.	Anti CCP ELISA,	4 days	5hrs	1hr	4 days (test done one in 4 days)
25.	ANA Profile	4 days	5hrs	1hr	4 days (test done one in 4 days)

26.	ANA ELISA	4 days	5hrs	1hr	4 days (test done one in 4 days)
27.	dS DNA ELISA	7days	5 hrs	1hr	Once in a week(Tuesday)
28.	Anticardiolipin ELISA	7 days	5hrs	1hr	Once in a week(Tuesday)
29.	Leptospira ELISA	4days	4hrs	1hr	Twice in a week (Wednesday, Saturday)
30.	Scrub Typhus ELISA	4 days	4hr	1hr	Twice in a week (Wednesday, Saturday)
31.	Anti pANCA /cANCA	7 days	5hrs	1hr	Once in a week (Friday)
32.	Complement (C 3) & Complement (C 4)	6days	24hr	1hr	Once in 7 days (Saturday)
33.	Protein C & S	6 days	6hr	1hr	Once in 7 days
34.	HBsAb titre by ELISA method	6days	5hr	1hr	Once in 7 days
35.	Endotoxin Chromogenic LAL assay	1hr	1day	1hr	1 day
36.	Hospital OT Surveillance (Aerobic &Anaerobic)	1hr	48 hrs for aerobic 7 days for anaerobic	1hr	48 hours for aerobic and 7 days for anaerobic organisms surveillance
37.	Disinfectant Testing	1hr	1 day	1hr	1 day
38.	Water samples & Dialysate testing	1hr	2days	1hr	2 days

Note: Viral marker samples received for ELISA after 8:30AM will be reported after 36 hours.

NOTE:-

1) All the OI/OP reports are available on HIS (SVIMS) online. Patients are requested to take the print outs from MRD section / Microbiology Reception.
2) All in-patients (IP) reports are available on HIS (SVIMS) online.
3) Microbiology department is Functioning 24 x7 x 365 days.
4) In case of sterile culture reports, final report will be available after 48 hrs. for all samples except urine, sputum and blood.
5) Urine and sputum with no significant bacterial growth, reports are available after 24 hours.

General instructions for all Tests/Samples:

a. **Outpatients:**

Sample Collection and performance of the test:

1. The requesting physician writes all the tests required in the case sheet of the patient and also fill a laboratory test requisition slip.
2. The Patient/ attendant pay the user charges for the prescribed test at the billing counter as per the requisition slip.
3. The billing clerk collects the user charges for the prescribed test and hands over the receipt to the concerned.
4. For clinical Microbiology investigations the patient visits the collection centre where his sample is collected and UHID no. is generated and pasted on the sample containers. All other necessary details are also pasted on the collection container. For Mycobacteriology, samples will be collected in OPD room no 44, DOTS centre.
   Details of sample collection are given in the manual.
5. Samples are received from collection centre to the clinical Microbiology laboratory till 4 pm. The samples are received at the reception counter. All details of the patient, requesting doctor, type of sample are entered in the flow register which is kept at the reception counter. The patient sample acceptance is also verifies in the hospital information system.

Any sample with incorrect or incomplete details is rejected:

6. The technician at the reception counter verifies all the details of patient and sample, and if found satisfactory delivers the sample to the working station. Patients are informed about the time, day and place for collecting the test reports for various tests.
7. In the lab the tests are performed following standard guidelines and reports are generated. All reports are verified, signed, dated clearly by authorized Microbiologist in charge of the specific section.
8. Reports are entered in the respective register of the sample and the reports are handed over to the personal assistant of the department to enter the reports in the hospital information system.
9. Patients/ relatives can collect the report from the reception area of the laboratory at the mentioned time and day. They may also collect the report from the MRD section of hospital (counter no. 3 & 4). The concerned doctor can see the reports on Personal computer in the OPD.

b. Inpatient:

Sample Collection and performance of test:

1. The requesting physician writes all the tests required in the case sheet of the patient and also fill a laboratory test requisition slip along with patient diagnosis/information regarding antimicrobial therapy, the same is also forwarded to the ward nurse.

2. The Patient/ attendant are informed about the investigations requested who in turn pays the charges for the prescribed test at the billing counter as per the requisition slip.

3. The billing clerk collects the charges for the prescribed test as per indicated in the laboratory test requisition slip and hands over the receipt of the billing.
4. The ward nurse collects the patient's sample, labels the sample with patient's name, age, sex, sample type, patients sample identification number as indicated in the laboratory test requisition slip.
5. The ward attendant carries the sample along with the test requisition form from the inpatient wards to the Microbiology lab.
6. Technician at the reception counter receives the samples, records the name of the patient, age, sex, sample type and patients sample identification number in the inpatient flow register.
7. Patients are informed about the time and day for collecting the test reports
8. In the lab the tests are performed following standard procedures, reports are generated. All reports are verified, signed, dated clearly by authorized Microbiologist in charge of the specific section.
9. Reports are entered in the respective register of the sample and the reports are handed over to the personal assistant of the department to enter the reports in the hospital information system.
10. Reports are dispatched to the concerned ward. The concerned doctor, ward nurse can also see the reports on PC in the hospital information website and they can take printout of it.
11. **Site of Report Collection:**
    a. All reports are available online in the hospital information system, Hospital Information Management System (HIMS).
    b. Outpatient investigation reports are handed over to the attendants/ patients at the report issue counter-Counter no. 3& 4 of MRD, SVIMS Padmavathi emergency & OPD building. Emergency reports will be available round the clock in department of Microbiology, at reception counter.

c. Inpatient reports are available in the HIMS for all IPD patients and they can take printout in respective wards/departments.

Samples hand delivered by clients, hospital staff or patient attendants to the laboratory:

- The individuals carrying the samples should first visit the sample reception counter (RoomNo.20 SVIMS Padmavathi emergency & OPD)

The test request form and suitability/quality of samples will be checked by laboratory staff and a provisional bill with the amount to be paid towards testing will be issued to the individual.

- The provisional bill amount has to be paid at the billing counter (Counter No. 3, Padmavathi OPD Block, SVIMS Padmavathi emergency & OPD) after which a printed invoice will be issued to the individual.

The printed invoice has to be produced immediately at the sample receipt counter for verification by laboratory staff.

- Physicians, clients or their representatives can opt to collect the report (hard copy) personally at the report issuing counter – at Counter no. 3& 4 of MRD, SVIMS Padmavathi emergency & OPD. Emergency reports will be available round the clock in department of Microbiology, at reception counter.

Samples transported by commercial courier services to the laboratory: NA

Sample Receipt timings:

Sample type	Collection centre	Location	Timings
OPD clinical Microbiology samples	OPD Collection centre	Room no.11 in Padmavathi OPD block	8am - 5 pm
	SPMCW Hospital	Ground floor next to registration counter	8 am - 1pm
	SVIMS clinic	SVIMS clinic building, sample collection centre no 17	7 am- 1pm
IPD Clinical Microbiology samples	samples from IP patients	Reception in the Department of Microbiology in SVIMS Padmavathi emergency & OPD building, Room no 20	24X7
Outside samples	All outside samples	Reception in the Department of Microbiology in SVIMS Padmavathi emergency & OPD building, Room no 20	24X7

Details of clinical samples to be sent for each test, type of container and the minimum quantity of sample to be sent for each test are tabulated on the following page (Sample Acceptance Criteria).

Sample acceptance/container/transport and storage

Specimen	Container	Patient Preparation	Special Instructions	Transportation to Laboratory	Storage before Processing	Primary Plating Media	Direct Examination	Comments
Abscess (also Lesion, Wound, Pustule, Ulcer)	Aerobic swab moistened with Stuart's or Amie's medium	Wipe area with sterile saline or 70% alcohol	Swab along leading edge of wound	< 2 hrs	24 hrs/RT	BA, CA, Mac, CNA optional	Gram	Add CNA if smear suggests mixed gram- positive and gram negative flora
Deep	Anaerobic transporter	Wipe area with sterile saline or 70% alcohol	Aspirate material from wall or excise tissue	< 2 hrs	24 hrs/RT	BA, CA, Mac, CNA Anaerobic BBA, LKV, BBE	Gram	Wash any granules and "emulsify" in saline
Blood or Bone Marrow Aspirate	Blood culture media set (aerobic and anaerobic bottle) or Vacutainer tube with SPS	Disinfect venipuncture site with 70% alcohol and disinfectant such as Betadine	Draw blood at time of febrile episode; draw two sets from right and left arms; do not draw more than three sets in a 24-hr period; draw ≥20 ml/set (adults) or 1-20 ml/set (pediatric) depending on patient's weight	Within 2 hrs/RT	Must be incubated at 37° C on receipt in laboratory	Blood culture bottles may be used. BA, CA BBAanaerobic	Direct gram Stain from positive blood culture bottles	Other considerations: brucellosis, tularemia, cell wall–deficient bacteria, leptospirosis, or AFB

Body Fluids Amniotic, abdominal, ascites (peritoneal), bile, joint (synovial), pericardial, pleural	Sterile, screw-cap tube or anaerobic transporter or direct inoculation into blood culture bottles	Disinfect skin before aspirating specimen	Needle aspiration	< 15 min	Plate as soon as received Blood culture bottles incubate at 37° C on receipt in laboratory	May use an aerobic and anaerobic blood culture bottle set for body fluids BA, CA, thio CNA, Mac (Peritoneal) BBA, BBE, LKV anaerobic	Gram (vaginal fluid is recommended)	May need to concentrate by centrifugation or filtration —stain and culture sediment
Bone	Sterile, screw-cap container	Disinfect skin before surgical procedure	Take sample from affected area for biopsy	Immediately/ RT	Plate as soon as received	BA, CA, Mac, thio	Gram	May need to homogenize
Cerebrospinal Fluid	Sterile, screw-cap tube	Disinfect skin before aspirating specimen	Consider rapid testing (e.g., Gram stain; cryptococcal antigen)	< 15 min	< 24 hrs Routine Incubate at 37° C except for viruses, which can be held at 4° C for up to 3 days	BA, CA (Routine) BA, CA, thio (shunt)	Gram—best sensitivity by cytocentrifugation (may also want to do AO if cytocentrifuge not available)	Add thio for CSF collected from shunt
Ear Inner	Sterile, screw-cap tube or anaerobic transporter	Clean ear canal with mild soap solution before	Aspirate material behind drum with syringe if	< 2 hrs	24 hrs/RT	BA, CA, Mac (add thio if prior antimicrobial	Gram	Add anaerobic culture plates for tympanocentesis specimens

		myringotomy (puncture of the ear drum)	ear drum intact; use swab to collect material from ruptured ear drum			therapy) BBA- (anaerobic)		
Outer	Aerobic swab moistened with Stuart's or Amie's medium	Wipe away crust with sterile saline	Firmly rotate swab in outer canal	< 2 hrs/RT	24 hrs/RT	BA, CA, Mac	Gram	
Eye Conjunctiva	Aerobic swab moistened with Stuart's or Amie's medium		Sample both eyes; use swab premoistened with sterile saline	< 2 hrs/RT	24 hrs/RT	BA, CA, Mac	Gram, AO, histologic stains (e.g., Giemsa)	Other considerations: Chlamydia trachomatis, viruses, and fungi
Aqueous/ vitreous fluid	Sterile, screw cap tube			< 15 min/RT	Set up immediately on receipt	BA, Mac, 7H10, Ana	Gram/AO	
Corneal scrapings	Bedside inoculation of BA, CA, SDA, 7H10, thio	Clinician should instill local anesthetic before collection		< 15 min/RT	Must be incubated at 28° C (SDA) or 37° C (everything else) on receipt in laboratory	BA, CA, SDA, 7H10, Ana, thio	Gram/AO The use of 10-mm frosted ring slides assists with location of specimen due to the size of the specimen	Other considerations: Acanthamoeba spp., herpes simplex virus and other viruses, Chlamydia trachomatis, and fungi

Foreign Bodies IUD	Sterile, screw-cap container	Disinfect skin before removal		< 15 min/RT	Plate as soon as received	Thio		
IV catheters, pins,	Sterile, screw-cap container	Disinfect skin before removal	Do not culture Foley catheters; IV catheters are cultured quantitatively by rolling the segment back and forth across agar with sterile forceps four times; ≥15 colonies are associated with clinical significance	< 15 min/RT	Plate as soon as received if possible store < 2 hrs 4° C	BA, Thio prosthetic valves		
GI Tract Gastric aspirate	Sterile, screw-cap tube	Collect in early AM before patient eats or gets out of bed	Most gastric aspirates are on infants or for AFB	< 15 min/RT	Must be neutralized with sodium bicarbonate within 1 hr of collection	BA, CA, Mac, HE, CNA, EB	Gram/AO	Other considerations: AFB
Gastric biopsy	Sterile, screw-cap tube (normal		Rapid urease test or culture for *Helicobacter*	< 1 hr/RT	24 hrs/4° C	BA, BBA	H&E stain optional: Immunostaining	Other considerations: urea breath test Antigen test

	saline < 2 hrs transport medium recomended)		*pylori*					(*H. pylori*)
Rectal swab	Swab placed in enteric transport medium		Insert swab ~ 2.5 cm past anal sphincter; feces should be visible on swab	Within 24 hrs/RT	< 48 hrs/RT or store 4° C	BA, Mac, XLD HE, Campy, EB	Methylene blue for fecal leukocytes	Other considerations: Vibrio, *Yersinia enterocolitica, Escherichia coli* O157:H7
Stool culture	Clean, leak-proof container; transfer feces to enteric transport medium (Cary-Blair) if transport will exceed 1 hr		Routine culture should include Salmonella, Shigella, and Campylobacter; specify Vibrio, Aeromonas, Plesiomonas, Yersinia, *Escherichia coli* O157:H7, if needed Follow-up may include Shiga toxin assay as recommened by CDC	Within 24 hrs/RT Unpreserved < 1 hr/RT	72 hrs/4° C	BA, Mac, XLD, HE, Campy, EB, optional: Mac-S; Chromogenic agar	Methylene blue for fecal leukocytes Optional: Shiga toxin testing	See considerations in previous rectal swabs Do not perform routine stool cultures for patients whose length of stay in the hospital exceeds 3 days and whose admitting diagnosis was not diarrhea; these patients should be tested for *Clostridium difficile*

O&P	O&P transporters (e.g., 10% formalin and PVA)	Collect three specimens every other day at a minimum for outpatients; hospitalized patients (inpatients) should have a daily specimen collected for 3 days; specimens from inpatients hospitalized more than 3 days should be discouraged	Wait 7-10 days if patient has received antiparasitic compounds, barium, iron, Kaopectate, metronidazole, Milk of Magnesia, Pepto-Bismol, or tetracycline	Within 24 hrs/RT	Indefinitely / RT		Liquid specimen should be examined for the presence of motile organisms with 30mins	
Genital Tract FEMALE Bartholin cyst	Anaerobic transporter	Disinfect skin before collection	Aspirate fluid; consider chlamydia and GC culture	< 2 hrs	24 hrs/RT	BA, CA, Mac, TM, Ana	Gram	
Cervix	Swab moistened with Stuart's	Remove mucus before collection	Do not use lubricant on speculum; use viral/	< 2 hrs/RT	24 hrs/RT	BA, CA, Mac, TM	Gram	

	or Amie's medium	of specimen	chlamydial transport medium, if necessary; swab deeply into endocervical canal					
Cul-de-sac	Anaerobic transporter		Submit aspirate	< 2 hrs/RT	24 hrs/RT	BA, CA, Mac, TM, Ana	Gram	
Endometrium	Anaerobic transporter		Surgical biopsy or transcervical aspirate via sheathed catheter	< 2 hrs/RT	24 hrs/RT	BA, CA, Mac, TM, Ana	Gram	
Urethra	Swab moistened with Stuart's or Amie's medium	Remove exudate from urethral opening	Collect discharge by massaging urethra against pubic symphysis or insert flexible swab 2-4 cm into urethra and rotate swab for 2 seconds;	< 2 hrs/RT	24 hrs/RT	BA, CA, TM	Gram	Other considerations: Chlamydia, Mycoplasma

			collect at least 1 hr after patient has urinated					
Vagina	Swab moistened with Stuart's or Amie's medium or JEMBEC transport system	Remove exudate	Swab secretions and mucous membrane of vagina	< 2 hrs/RT	24 hrs/RT	BA, TM Culture is not recommended for the diagnosis of bacterial vaginosis; inoculate selective medium for group B Streptococcus (LIM broth) if indicated on pregnant women	Gram	Examine Gram stain for bacterial vaginosis, especially white blood cells, clue cells, grampositive rods indicative of Lactobacillus, and curved, gramnegative rods indicative of Mobiluncus spp.
MALE Prostate	Swab moistened with Stuart's or Amie's medium or sterile, screw-cap tube	Clean glans with soap and water	Collect secretions on swab or in tube	< 2 hrs/RT for swab; immediately if in tube/RT	Swab: 24 hrs/ RT; tube: plate secretions immediately	BA, CA, Mac, TM, CNA	Gram	

Urethra	Swab moistened with Stuart's or Amie's medium or JEMBEC transport system		Insert flexible swab 2-4 cm into urethra and rotate for 2 seconds or collect discharge on JEMBEC transport system	< 2 hrs/RT for swab; within 2 hrs for JEMBEC system	24 hrs/RT for swab; put JEMBEC at 37° C immediately on receipt in laboratory	BA, CA, TM	Gram	Other considerations: Chlamydia, Mycoplasma
Hair, Nails, or Skin Scrapings (for fungal culture)	Clean, screw-top tube	Nails or skin: wipe with 70% alcohol	Hair: collect hairs with intact shaft Nails: send clippings of affected area Skin: scrape skin at leading edge of lesion	Within 24 hrs/RT	Indefinitely/RT	SDA, IMAcg, SDAcg	CW	
Respiratory tract LOWER BAL, BB, BW		Sterile, screw-top container	Anaerobic culture appropriate only if sheathed (protected) catheter used	< 2 hrs/RT	24 hrs/4° C	BA, CA, Mac, CNA	Gram and other special stains as requested (e.g., Legionella DFA, acid-fast stain)	Other considerations: quantitative culture for BAL, AFB, Legionella, Nocardia, Mycoplasma, Pneumocystis, cytomegalovirus

Sputum, tracheal aspirate (suction)	Sterile, screw-top container	Sputum: have patient brush teeth and then rinse or gargle with water before collection	Sputum: have patient collect from deep cough; specimen should be examined for suitability for culture by Gram stain; induced sputa on pediatric or uncooperative patients may be watery because of saline nebulization	< 2 hrs/RT	24 hrs/4° C	BA, CA, Mac PC OFPBL-cystic fibrosis	Gram and other special stains as requested (e.g., Legionella DFA, acid-fast stain)	Other considerations: AFB, *Nocardia*
UPPER Nasopharynx Nose	Swab moistened with Stuart's or Amie's medium		Insert flexible swab through nose into posterior nasopharynx and rotate for 5 seconds; specimen of choice for Bordetella pertussis	< 2 hrs/RT	24 hrs/RT	BA, CA BA, chromogenic agar		Other considerations: add special media for *Corynebacterium diphtheriae*, pertussis, *Chlamydia*, and *Mycoplasma*

Pharynx (throat)	Swab moistened with Stuart's or Amie's medium		Swab posterior pharynx and tonsils; routine culture for group A Streptococcus *(S. pyogenes)* only	< 2 hrs/RT	24 hrs/RT	BA or SSA		Other considerations: add special media for *C. diphtheriae, Neisseria gonorrhoeae*, and epiglottis *(Haemophilus influenzae)*
Tissue	Anaerobic transporter or sterile, screw-cap tube	Disinfect skin	Do not allow specimen to dry out; moisten with sterile, distilled water if not bloody	< 15 min/RT	24 hrs/RT	BA, CA, Mac, CNA, Thio Anaerobic: BBA, LKV, BBE	Gram	May need to homogenize
Urine Clean-voided midstream (CVS)	Sterile, screw-cap container Containers that include a variety of chemical urinalysis preservatives may also be used	Females: clean area with soap and water, then rinse with water; hold labia apart and begin voiding in commode; after several mL have passed, collect midstream		Preserved within 24 hrs/RT unpreserved < 2 hrs/RT	24 hrs/4° C	BA, Mac Optional: Chromogenic agar	Check for pyuria, Gram stain not recommended	Plate quantitatively at 1 : 1000; consider plating quantitatively at 1 : 100 if patient is female of childbearing age with white blood cells and possible acute urethral syndrome

		Males: clean glans with soap and water, then rinse with water; retract foreskin; after several mL have passed, collect midstream						
Straight catheter (in and out) and rinse (water)	Sterile, screw-cap container	Clean urethral area (soap and water)	Insert catheter into bladder; allow first 15 mL to pass; then collect remainder	< 2 hrs/RT preserved < 24 hrs/RT	24 hrs/4° C	BA, Mac	Gram or check for pyuria	Plate quantitatively at 1 : 100 and 1 : 1000
Indwelling catheter (Foley)	Sterile, screw-cap container	Disinfect catheter collection port	Aspirate 5-10 mL of urine with needle and syringe	< 2 hrs/4° C (preserved < 24 hrs/RT)	24 hrs/4° C	BA, Mac	Gram or check for pyuria	Plate quantitatively at 1 : 1000
Suprapubic aspirate	Sterile, screw-cap container or anaerobic transporter	Disinfect skin	Needle aspiration above the symphysis pubis through the abdominal wall into the full bladder	Immediately/RT	Plate as soon as received	BA, Mac, Ana, Thio	Gram or check for pyuria	Plate quantitatively at 1 : 100 and 1 : 1000

7H10, Middlebrook 7H10 agar; *AFB*, acid-fast bacilli; *AM*, morning; *Ana,* anaerobic agars as appropriate (see Chapter 41); *AO*, acridine orange stain; *BA*, blood agar; *BAL*, bronchial alveolar lavage; *BB*,bronchial brush; *BBA*, brucella blood agar; *BBE*, Bacteroides bile esculin agar; *BW*, bronchial wash; *CA*, chocolate agar; *Campy*, selective Campylobacter agar; *CNA*, Columbia agar with colistin and nalidixic acid; *CW*, calcofluor white stain; *DFA*, direct fluorescent antibody stain; *EB*, enrichment broth; *GC*, *Neisseria gonorrhoeae*; transport using JEMBEC system with modified Thayer-Martin; *GI*, gastrointestinal; *Gram*, Gram stain; *HBT*, human blood-bilayer Tween agar; *HE*, Hektoen enteric agar; *hrs*, hours; *IMAcg*, inhibitory mold agar with chloramphenicol and gentamicin; *IUD*, intrauterine device; *LKV*, laked blood agar with kanamycin and vancomycin; *Mac*, MacConkey agar; *Mac*-S, MacConkey-sorbitol; *mL*, milliliters; *OFPBL*, oxdative-fermentative polymixin B-bacitracin-lactose-agar; *O&P*, ova and parasite examination; *PC*, Pseudomonas cepacia agar; *PVA*, polyvinyl alcohol; *RT*, room temperature; *SDA*, Sabouraud dextrose agar; *SDAcg*, Sabouraud; dextrose agar with cycloheximide and gentamicin; *SPS*, sodium polyanethol sulfonate; *SSA*, group A streptococcus selective agar; *thio*, thioglycollate broth; *TM*, Thayer-Martin agar; *XLD*, xylose lysine deoxycholate agar. *Specimens for viruses, chlamydia, and mycoplasma are usually submitted in appropriate transport media at 4° C to stabilize respective microorganisms.

Mycology specimen collection

S. No	Specimen	Collection	Unacceptable specimen
1	Pus	Aseptically with needle and syringe from undrained abscess. Pus expressed from abscess opened with scalpel; transported to laboratory either in sterile container/ syringe and needle	Swab or materials from open wound
2	Biopsy	Place between two sterile gauze pads, sterile petri dish/ tube (containing 2-3 mL of sterile normal saline/ brain heart infusion broth) Tissue is collected from centre and edge of the lesion.	Swabs, samples collected in thioglycolate broth or normal saline
3	Grains	Collected by lifting the crust at the opening of a sinus. Grains frequently found underneath the pus or collected from the removed bandages Aspirated from undrained sinuses	
4	Cerebrospinal fluid	3 mL in a sterile tube	Insufficient quantity
5	Body fluids	Sterile tube or in a heparinized syringe	Swabs
6	Bone marrow	0.2-0.3 mL collected in a sterile heparinized syringe Sterile cap is placed on heparinized syringe and transported immediately	Clotted bone marrow
7	Blood	5-10 mL in yellow Vacutainer/syringe or in biphasic media containing brain heart infusion broth and agar; blood: broth ratio should be maintained at 1:10. Multiple blood cultures at timed intervals to be collected BACTEC/lysis centrifugation technique may improve sensitivity	
8	Urine	Early morning 25-50 mL of clean catch midstream urine specimen Suprapubic aspirate, catheterized specimen Collected in sterile container	24 hours collection is unacceptable
9	Faeces	Not usually acceptable in the mycology laboratory. Sometime collected to access *Candida* carriage in GI tract.	
10	Sputum	5-10 mL; early morning prior to eating Use mouth rinse and brush before collection Collected in sterile wide mouthed container.	Saliva, nasal secretion, throat swab, 24 hour collection
11	Bronchial brush/washing/ broncho alveolar lavage	Collected in sterile container using fiber optic bronchoscopes	Dried specimen

12	Lung biopsy •	• Collected by bronchoscope, • fluoroscope guided trans-thoracic needle aspiration or open lung biopsy • Best specimen is open lung biopsy but it is hazardous	
13	Serology	•Serum: 1-2 mL • 3-5 mL of spinal fluid	Specimen collected after skin test with histoplasmin while performing serology for histoplasmosis

Transport of specimens

• Specimens should be transported in sterile, humidified, leak-proof container. Dermatological specimens, however, should be transported in a dry container. Transport medium should not be used unless the specimen can be easily and completely retrieved from the medium. Although fungi can be recovered at times from specimens submitted in anaerobic transport media, such media should be avoided.

• Specimens should be processed and inoculated to primary isolation media as soon as possible after collection, ideally within few hours. It should not be presumed that successful methods for storage of fungal cultures are suitable for temporary storage of clinical specimens that harbour relatively few fungal cells.

• The effect of refrigeration on fungal specimens has not been well studied, but if processing is to be delayed for more than several hours, it is recommended that specimens be stored under refrigeration at 4°C with the following exceptions: blood and cerebrospinal fluid are stored at 30-37°C (some fastidious organism may not survive at 4°C) dermatological specimens are stored at 15-30°C (dermatophytes survival is best at this temperature).

Serology, immunology and Molecular Microbiology – Serum

The phlebotomists confirm the identity of the patient by checking the full name of the patient with initials and confirming the details with the patient and checking the registration number. Following patient identification, the phlebotomists explains the blood drawing procedure to the subject and reassures him/her for safety.

Serum sample collection & transport

Tube type	Colour	volume	Tests to be performed
Serum Tube with or without clot activator gel	Plastic or Glass - Red	3ml	Serology, immunology and Molecular Microbiology

After the requested tests are performed, the remaining volume of Serum/CSF samples will be stored at -80^{0}C for 7 days, after which they will be discarded.

COLLECTION OF STOOL SPECIMEN:

Collect the stool in a sterile wide mouthed container with a tight fitted lid without contamination of urine, water or disinfectants.

Ideally 3 faecal samples are advised to diagnose intestinal parasitic diseases. Two samples on successive days during normal bowel movement. 3rd sample after magnesium purge.

Sample should be sent immediately to the lab without delay after collection or refrigerated until transport is possible.

Ideally 30 minutes for liquid specimens, semiformed stool within 1 hour for suspected infection like E.histolytica and G.lamblia.

Clinician must specify the diagnosis on the requisition form and to be sent along with stool sample.

All faecal specimens should be collected prior to the administration of antibiotics or antidiarrhoeal agents.

Use of mineral oil, bismuth and barium prior to faecal collection should be avoided.

Stool samples are examined by saline and iodine wet mount for parasitic eggs,cysts,trophozoits and adult worms.

Stool samples for Cryptosporidium, Cyclospora and Isospora are examined by modified acid fast staining.

SAMPLE REJECTION CRITERIA FOR STOOL SAMPLES:

1. Dried specimens

2. Delayed specimens

3. Sample collected in a leaky and unsterile container

4. Samples contaminated with urine ,water and disinfectants.

SAMPLE REJECTION CRITERIA:
Events outside the laboratory:

Code no.	Description
1	**ERRORS IN REQUISITION FORMS**
a	Missing/incomplete data on the request - no diagnosis, no clinician's/ requesting personnel signature
b	Bar code is not legible due to poor quality of print out
c	Requisition forms with hand written test parameters which are not confirmed
d	Sample sent without requisition form
e	Requisition form received without sample
f	Sample collection not confirmed and test not displayed in lab system for the patient
2	**SAMPLE LABELLING ERRORS**
a	Incorrect patient identification -improper labelling- mismatch
b	Labels not properly pasted on sample container - falling off and replaced by worker transporting the samples
3	**SAMPLE ERRORS**
a	Sample type mismatch- urine sample received for CSF request, etc
b	Specimen insufficient
c	Samples intended for other labs received - sample mixup
d	Delayed/improper transportation of sample
e	Haemolysed samples
f	Leakage of sample from container
g	contaminated sample ex: sputum sample contaminated with saliva

Events within the laboratory:

Code no.	Description
4	Upon separation serum sample is found to be hemolyzed - improper sample collection, transportation, centrifugation
5	Sample mix up while transferring sample from collection tube to centrifuge tube and from centrifuge tube to sample cups for analyzer.
6	Sample loss due to tube breakage in centrifuge and or spillage

Analysis of samples:

Procedures and methods that are up to date with current practices according to department SOP manual will be used.

All procedures are performed in accordance with strict qualitv control by authorized personnel.

The tests are periodically evaluated using internal quality control as well as external quality assurance.

Release of laboratory reports

Hard copies of reports if required can be collected personally by clients/patients at the report issue counter (Counter no. 3& 4 of MRD, SVIMS Padmavathi emergency & OPD building)

Reports will NOT be sent by postal or courier services or by email.

Reports over telephone are usually avoided except for emergency situations/under special circumstances. In such situations the report will be conveyed only to treating physician who is in charge of that particular case.

All critical values will be informed to the concerned wards immediately along with hospital information system update of concerned report.

Retention of specimens after reporting:

- Pus and body fluids samples are discarded after 72 hours of release of reports.
- Urine and sputum samples discarded after 24 hours
- CSF and Peritoneal dialysis fluid samples will be discarded after 5 days of release of results.
- Serum samples for serology & immunology tests will be discarded after one week of completion of the test.

PROCEDURES FOR SAMPTE COLLECTION:

BLOOD

Prepare the site

- Select the site of venipuncture. If the patient is unusually dirty, wash the intended site withsoap and water prior to venipuncture.
- Apply a tourniquet, 3-4 inches above the intended site of venipuncture. Alternatively this
  can be done after cleaning.
- Put on examination gloves.
- Vigorously cleanse with 70% isopropyl or ethyl alcohol to remove surface dirt and oils. Scrub the venipuncture site gently but firmly with the cotton beginning in the center and continuing in an outward direction circularly for an area of 4 to 5 inches in diameter.
- Allow to dry.
- Swab or wipe concentric circles of 2% w/v chlorhexidine with 70% isopropyl alcohol or 10% w/v povidone iodine/tincture of iodine, in a similar manner as given earlier- beginning in the center and continuing in an outward direction circularly for an area of 4 to 5 inchesin diameter.
- Allow the povidone iodine to dry (2 minutes). For chlorhexidine gluconate (2%w/v)/ tincture I (10%w/v), drying period is ~ 30 seconds.
- Do NOT touch the site after cleaning.
- Instruct patient to clench and unclench the fist.
- Perform phlebotomy using the needle and syringe.
- Release the tourniquet and withdraw the needle.
- Apply pressure to the site of venipuncture and place a bandage over the puncture site.
- Skin preparation with either alcohol, alcoholic chlorhexidine (2% w/v), or tincture of iodine (10% w/v) leads to lower blood culture contamination rates than does the use of povidone-iodine[6,7].

For pediatric patients

- **< 2months:** Omit the iodine step, and clean two additional times with separate preparation pads saturated with 70% isopropyl alcohol or ethyl alcohol
- **> 2 months:** Chlorhexidine gluconate as a skin antiseptic is approved for use in pediatricpatients two months of age and older[9].

Prepare the bottle

Prepare the septum of the blood culture bottle and the rubber stoppers on bottles or tubes. Label thebottles with the patient's name and the date and time of draw.

Site of draw may be listed.

Collection through an intravenous line

- It is not necessary to discard the initial volume of blood or flush the line with saline to
  eliminate residual heparin or other anticoagulants[9].
- Vigorously wipe septa with 70% alcohol and allow drying completely, for 30 to 60 seconds.
- Pediatric bottles should not to be used for adult patients except for those elderly patients in whom it's difficult to obtain larger amounts of blood.
- **Adult patient (50 kg):** 10-20 ml, divided between **two** blood cultures from **separate**,peripheral venipuncture **sites**.
- Anerobic blood cultures should be taken only if there are adequate resources[10].
- **Pediatric patient:** 6-10 ml, divided between two blood cultures.
- Initially obtain **three** blood culture sets within a **30 minute** period **before administration of empiric antimicrobial agents** from patients presenting with possible **infective endocarditis**. If those sets are negative **at 24 hours**, obtain two more sets of cultures, fora total of five sets overall[9].

Transport of blood culture bottles

In case of delay between collection and processing, **never refrigerate the bottle**. Preferably keep the bottle in a 35ºC incubator, if available. Otherwise, leave the bottle at room temperature.

CSF

- Cap, face mask, gown and gloves for physician drawing CSF are useful adjuncts to infection prevention. Disinfect the puncture site with antiseptic solution and alcohol in a manner identical to phlebotomy skin preparation for blood culture to prevent specimen contamination and introduction of infection.
- Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace. When the subarachnoid space is reached, remove the stylet; spinal fluid will appear in the needle hub.

  Measure the hydrostatic pressure with a manometer

Specimen transport

- Submit to laboratory as soon as possible and alert laboratory that specimen is in transit.
- Do not refrigerate.
- Each sterile calibrated tube containing CSF must be properly labeled with the patient's name, unique identification number, and the date and time of collection.
- Requisition must be complete with demographic and specimen collection information.Record the patient diagnosis for proper processing of specimen.

BODY FLUIDS FROM STERILE SITES

Specimen collection

- Body fluids from sterile sites should be collected by percutaneous aspiration for pleural, pericardial, peritoneal, amniotic, and synovial fluids.
- Use care to avoid contamination with commensal microbiota.
- Clean the needle puncture site with alcohol, and disinfect it with an iodine solution [1- 2% tincture of iodine or a 10% solution of povidone iodine (1% free iodine)] to prevent specimen contamination or infection of patient (if tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn).
- Aseptically perform percutaneous aspiration with syringe and needle to obtain pleural,
- pericardial, peritoneal, or synovial fluid. Use safety devices to protect from

needle exposure.

- Immediately place a portion of the joint fluid or peritoneal fluid collected from patients with CAPD or SBP into aerobic and anaerobic blood culture bottles, retaining some (0.5 ml) in syringe for Gram stain and direct plating.
- Use the minimum and maximum volumes recommended by the bottle manufacturer
  (generally up to 10 ml is the maximum for each bottle).
- Alternatively, inoculate the blood culture bottles after receipt in the laboratory.
- Submit other fluids and the remainder of specimens after inoculation of blood culture bottles in one of the following: a sterile, gassed-out tube or a sterile blood collection tube without preservative; however, fluids in such tubes may clot during transport.

Specimen transport

- Submit to laboratory as soon as possible and, if from a normally sterile site, alert laboratorythat specimen has been submitted.
- Do not refrigerate.
- Label specimens with patient demographics and date, time, and site of collection,*e.g*. left
  knee joint fluid.

  Record the patient diagnosis for improved processing of specimen

Note:

- If specimens inoculated into blood culture bottles are received, Gram stain cannot beperformed.
- Collect specimen prior to antimicrobial therapy for greatest diagnostic sensitivity.
- Do not submit specimens from drains after they have been infused with antimicrobialagents.
- Call physician when fluid specimens are received on a swab.
- Contact physician if specimen is insufficient for the number of tests requested.
- Swabs constitute the least desirable sample for culture of body fluids and should be discouraged, since the quantity of sample may not be sufficient to ensure recovery of a small number of organisms.

- Routine bacterial culture is sufficient for culture for *Candida* species, if blood culture

  bottles are used or specimen is centrifuged.

Important considerations

- Specimens received by the laboratory in a syringe with the needle still attached should be rejected because of the risk of a needless sharp exposure by laboratory staff. The physician should be immediately contacted to recollect the sample and send it in proper container. **Note:** Establish a policy for the proper collection and transport of clinical specimens not collected on swabs. Educate the physicians that needles must be removed from the syringe and the syringe cap secured prior to transport to avoid leakage.
- Syringes that have been capped with a Luer-Lock (with needle removed) prior to transport may be accepted for culture provided the specimen has not clotted inside the syringe and there is no leakage during transport which could result in contamination of the culture. The laboratory may reject specimens that have clotted in a capped syringe because they cannot be processed for culture without inadvertently contaminating the specimen.

OCULAR SPECIMENS

Specimen collection and transport

Note: Most eye specimens should be collected by an ophthalmologist. These specimens should be inoculated onto culture media at the bedside, in the clinic or the physician's office. A variety of techniques are used to collect material from different parts of the eye. The conjunctiva is constantly contaminated by various bacteria from the environment and ocular adnexa. Therefore, specimens from the conjunctiva serve as a control when compared with specimens collected by more aggressive or invasive techniques.

Considerations

- Provide fresh media to the clinical areas routinely collecting ocular cultures, and instruct physicians to immediately transport inoculated media and slides to the laboratory.
- Obtain viral and chlamydial samples before topical anesthetics are instilled.
- Obtain samples for chlamydial cultures with calcium alginate swabs.
  Note: Calcium alginate swabs may be toxic for *Neisseria gonorrhoeae* (for which rayonor cotton swabs could be used) [17].
- For viral cultures, use Dacron or cotton swabs with non-wood shafts[18].

Collection by anatomic site[16]

Conjunctiva (bacterial conjunctivitis) and lid margin (blepharoconjunctivitis)

- Obtain the specimen with a sterile, pre-moistened cotton or calcium alginate swab.
- Roll the calcium alginate or cotton swab over the conjunctiva before topical medicationsare applied.
- Culture both eyes with separate swabs.
- Immediately inoculate the material at the bedside onto BAP and CHOC.
- Inoculate the swab from the right conjunctiva in horizontal streaks, and inoculate the swab from the left conjunctiva in vertical streaks, each on one half of the same agar plate.
- Inoculate specimens from the right and left lid margins, if collected, by

making an R and an L to represent the respective sites on another agar plate.

- Obtain conjunctival scrapings for a smear preparation as follows:
- Instill 1 or 2 drops of proparacaine hydrochloride.
- Using a Kimura spatula, gently scrape across the lower right tarsal conjunctiva.
- Smear the material in a circular area 1 cm in diameter on a clean glass slide.
- Prepare at least two slides.
- Immerse the slides in 95% methyl alcohol or 95% methanol for 5 minutes.
- Repeat steps for the left conjunctiva.

Cornea (bacterial keratitis)

- Instill 1 or 2 drops of proparacaine hydrochloride (local anesthetic for ophthalmic instillation).
- Obtain conjunctival samples as described above, and then obtain corneal scrapings from

  the advancing edge of the ulcer by scraping multiple areas of ulceration and suppuration with a sterile Kimura spatula, using short, firm strokes in one direction (keep the eyelid open, and be careful not to touch the eyelashes).
- Obtain approximately three to five scrapings per cornea.
- Inoculate each set of scrapings onto BAP and CHOC, using a 'C' formation for eachscraping.
- Prepare smears by applying the scrapings in a gentle circular motion over a clean glass

  slide or by compressing material between two clean glass slides and pulling the slidesapart.

Bacterial endophthalmitis

- Collect an aspirate of the vitreous fluid or perform a paracentesis of the anterior chamber using a needle aspiration technique to collect intraocular fluid.
- Collect specimens for conjunctival cultures along with the fluid to determine the significance of indigenous microbiota.
- If a small volume of fluid is collected, inoculate cultures at the bedside by inoculating 1 or2 drops of fluid onto culture media.

RESPIRATORY SPECIMENS

Specimen collection and transport

Sputum

- Spontaneous: Early morning specimen generated after a bout of cough.
- Having the patient brush his or her teeth and gargle with water immediately before obtaining the sputum specimen reduces the number of contaminating oropharyngeal bacteria.
- Collect specimen resulting from deep cough in a sterile screw-cap cup or other suitable sterile collection assembly of about 100 ml capacity.
- To prevent contamination of the outside of the container, the patient should be instructed to press the rim of the container under the lower lip to catch the entire expectorated coughsample.
- Tightly screw on the cap of the container. Wipe off any spilled material on its outside with a tissue moistened with disinfectant, but take care not to let any disinfectant enter the container. Such communication with patients can be rewarding. In addition, patients should remove dentures during the specimen collection.
- Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Twenty four hour collections should be discouraged[6,7,19].
- Deliver the specimen to the laboratory as quickly as possible, preferably within 2 hours, for delicate bacterial, viral and mycoplasma pathogens may die out during longer delay.

Endotracheal aspirate (ETA) [17]

- Endotracheal aspiration should be done with a sterile technique using a 22 inch, 12F suction catheter. The catheter should be introduced through the endotracheal tube for at least 30 cm. Gentle aspiration is then performed without instilling saline solution. The firstaspirate is discarded.
- The second aspirate should be collected after tracheal instillation of 5 ml saline in a mucus collection tube. [If very little secretion is produced by the patient, chest vibration or percussion for 10 minutes should be used to increase the retrieved

volume (≥ 1 ml)].

- The specimens should be sent to laboratory and cultured within 1 hour of collection.

Bronchoalveolar lavage (BAL) [21]

In this procedure 120 ml of saline should be infused into a lung segment through the bronchoscope to obtain cells and protein of the pulmonary interstitium and alveolar spaces. Send a portion of it to the laboratory.

Sinus aspirate

Collection of specimens from patients with sinusitis should be performed by otolaryngologists who perform nasal endoscopy or sinus puncture and aspiration.

Rejection criteria

For sputum and endotracheal aspirate specimens

- Reject duplicate specimens received on the same day unless the initial sample wasinappropriate for culture according to microscopic evaluation.
- Do not accept repeat cultures at intervals of less than every 48 hours.
- Reject the following specimens for diagnosis of lower respiratory tract disease:
  - 24 hours sputum collection
  - Contaminated sputum and endotracheal specimens as per Gram stain rejection criteria(see below)
  - Specimens that are visually saliva only
  - Specimens that are visibly contaminated with toothpaste or other substances
  - Nasal washes or swabs of nares to diagnose sinusitis
- Sputum samples are highly contaminated with normal anaerobic flora of the upper

  respiratory tract. Therefore, anaerobic culture should not be done.

For BAL, lung aspirates and OLB specimens

- BAL specimens, lung aspirates, and OLB specimens should never be rejected by the laboratory, since the patient has undergone an invasive procedure for their collection.

- For specimens delayed in transit more than 2 hours without refrigeration, indicate on the report that the delay in transit may compromise the culture results.
- Anaerobic culture should be performed on lung aspirates, pleural fluid, and OLB specimens by request or when the original specimen Gram stain demonstrates morphotypes suggestive of anaerobic infection.

PUS

Specimen collection

- Preferably collect specimen prior to initiation of therapy and only from wounds that are clinically infected or deteriorating or that fail to heal over a long period.
- Cleanse surrounding skin or mucosal surfaces.
- For closed wounds and aspirates, disinfect with 2% chlorhexidine or 70% alcohol followed by an iodine solution [1 to 2% tincture of iodine or a 10% solution of povidone-iodine (1% free iodine)]. Remove iodine with alcohol prior to specimen collection.
- For open wounds, debride, if appropriate, and thoroughly rinse with sterile saline prior to
  collection. Sample viable infected tissue, rather than superficial debris.

Wound or abscess aspirates

- Samples collected by using a syringe and needle should be placed in a sterile container or blood collection tube without anticoagulant (*e.g.*, Vacutainer® or similar type) for submission to the laboratory.
- A portion of the sample should also be placed in a sterile tube containing anaerobic medium
  like RCM if an anaerobic culture is required.

Open wounds

- Cleanse the superficial area thoroughly with sterile saline, changing sponges with eachapplication. Remove all superficial exudates.
- Remove overlying debris with scalpel and swabs or sponges.

- Collect biopsy or curette sample from base or advancing margin of lesion.

Pus

- Aspirate the deepest portion of the lesion or exudate with a syringe and needle.
- Collect a biopsy sample of the advancing margin or base of the infected lesion afterexcision and drainage.
- For bite wounds, aspirate pus from the wound, or obtain it at the time of incision, drainage,
  or debridement of infected wound.

Tissues and biopsy samples

- Tissue biopsy samples should be collected from areas within and adjacent to the area of infection. Large enough tissue samples should be collected to perform all of the tests required (i.e., 3 to 4 mm biopsy samples).
- If anaerobic culture is required, a separate piece of tissue should be submitted in a steriletube containing anaerobic medium like RCM.
- Collect swabs only when tissue or aspirate cannot be obtained.
- Limit swab sampling to wounds that are clinically infected or those that are chronic andnon-healing.
- Remove superficial debris by thorough irrigation and cleansing with non-bacteriostatic sterile saline. If wound is relatively dry, collect with two cotton-tipped swabs moistenedwith sterile saline.
- Gently roll swab over the surface of the wound approximately five times, focusing on area where there is evidence of pus or inflamed tissue.

Note: Organisms may not be distributed evenly in a burn wound, so sampling different areas of theburn is recommended. Blood cultures should be used to monitor patient status.

Standard precautions to be followed while handling the specimen

Note: Syringes with the needle attached should not be accepted due to the sharps and biohazard riskto staff.

- Grossly contaminated specimen or leaky containers and collection containers of doubtfulsterility must be noted and mentioned.
- Deliver aspirates and tissues to the laboratory within 30 minutes for best recovery.
- Keep tissues moist to preserve organism viability.
- Do not refrigerate or incubate before or during transport. If there is a delay, keep sample at room temperature, because at lower temperature there is likely to be more dissolved oxygen, which could be detrimental to anaerobes.

Rejection criteria

- For anaerobic culture, avoid swab collection if aspirates or biopsy samples can be obtained**.**

Do not accept specimens for microbiological analysis in container with formalin

URINE

Collection of urine

Midstream clean catch urine

- The midstream clean catch urine is the most common type of urine specimen.
- The technique involved in collection is based on voiding the first portion of urine, which
  is most likely to be contaminated by urethral commensals.
- It is recommended that the first voided morning specimen be collected, as bacteria would
  have multiplied to high levels after overnight incubation in the bladder.
- If not possible, the urine can be collected during the day, preferably 4 hours after the last
  void, keeping in mind that the counts may be lower, yet significant.

- Midstream clean catch urine should be collected in a sterile, wide mouth, screw capped bottle after very thorough preliminary cleaning of external genitalia with soap and water. Antiseptics should not be used for this purpose.

Indwelling catheter

- Hospitalized patients with indwelling catheter are especially at risk of developing UTI.
- To avoid contamination, the specimen should be collected by disinfecting a portion of the catheter tubing with alcohol & puncturing the tubing directly with a sterile syringe with needle and aspirating the urine.
- The urine MUST NOT be collected from the drainage bag.

Suprapubic collection

- The suprapubic collection avoids urethral contamination but is invasive.
- This procedure is usually reserved for infants and adults, from whom it is difficult to
  obtain a midstream clean catch urine specimen.
- Disinfect the skin above the bladder and plunge a sterile needle with syringe into the
  bladder; aspirate the urine and transfer to a sterile container.

Percutaneous nephrostomy (PCN) aspirate

- Percutaneous nephrostomy aspirate is urine collected directly from renal pelvis.
- If the sample is a PCN catheter sample, collection must be done as explained for indwellingcatheters and not from the drainage bag.

Cystoscopy specimens

- Cystoscopy specimen is urine collected from the bladder during cystoscopy.

Ileal conduit specimen

- Ileal conduit specimen is collected after cleaning stoma site.
- A fresh drain of urine is collected. It must not be collected from the urine drainage bag.

Intermittent catheter specimen

- A red rubber catheter should be introduced into the urethra periodically to drain urine fromthe bladder.
- It should be collected directly into a specimen container.

Specimen Transport

- Urine must be transported to the lab as soon as possible.
- It should be cultured as early as possible after collection, preferably within 2 hours.
- In case of delay, it may be refrigerated up to a maximum of 24 hours before plating.

FECAL SPECIMENS

Specimen collection and transport

- A small quantity of solid/semisolid stool or one third of the container in case of watery stool is collected in a sterile screw-capped disposable 40 ml container.
- A rectal swab is not recommended as the material obtained is never adequate for all the
  tests or for inoculating all the media used for culture.
- The sample should be collected preferably prior to initiation of antibiotics in the container directly, taking care not to soil the outside of the container. Samples should not be collectedfrom bedpan.
- The sample should be immediately transported to the laboratory on collection.
- If there is a delay in transporting faecal specimens or if samples need to be sent by post, one of the following transport media may be employed:
  - ♦ Phosphate buffered glycerol saline solution
  - ♦ Stuart's transport medium
  - ♦ Cary and Blair transport medium

TISSUE SAMPLE PROCESSING

Grind or homogenise specimen with appropriate instruments or equipments, using a sterile tissuegrinder (beads), a sterile scalpel or sterile scissors and petri dish.

- A small volume (approximately 0.5mL) of sterile, saline/water, peptone or broth should beadded to aid the homogenisation process.
- Ideally, all grinding or homogenisation should be performed in a Class II exhaust protective cabinet.

  Note: Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenized

  Select media, incubation times, temperature and other features following SOP like pus/aspirates.

References:

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3. https://main.icmr.nic.in/sites/default/files/guidelines/Mycology_SOP_2nd_Ed_2019.pdf