

Department of Microbiology
Urine Specimen Processing Procedures



Test Name & Order Code	Specimen Types	Media & Inoculation	Incubation
Culture Urine, No Smear CURNNS	Clean catch, Catheter (closed or foley), Ileo-conduit, Pedi-bag urines (including suprapubic bags), Nephrostomy	CHROMagar Orientation/BAP Use 0.001 mL (small) calibrated loop	Ambient at 35 ± 2 °C
Culture Urine, No Smear CURNNS	Straight cath, Cystoscopy kidney, Suprapubic aspirate, VB1 VB2 VB3 prostatic urines, Prostatic Secretions, Cystoscopy bladder, PCN (percutaneous nephrostomy)	CHROMagar Orientation/BAP Use 0.001 mL (small) calibrated loop. BAP (whole) Use 0.01 mL (large) calibrated loop to inoculate.	Ambient at 35 ± 2 °C
Test Name & Order Code	Comments	Specimen Processing	
Gram Stain Smear GSSM	Must be ordered separate from culture.	Use 0.01 mL (large) loop to transfer urine to glass slide etched with circle.	
Culture Yeast CYEST	Urine submitted with a CFC order should be submitted for change to CYEST	CHROMagar <i>Candida</i> (date plate) • Use 0.001 mL (small) cal. loop Gram Stain • Use 0.01 mL (large) cal. loop	Ambient at 35 ± 2 °C (incubate in the dark)
Culture Trichomonas CTRICH	Urine may be submitted for inpatients only. PAML clients must submit all specimens in the InPouch TV device.	Process urine w/in 30 min of collection. 15 mL of fresh urine from <u>male</u> patient. Centrifuge at 500 rpm for 5 min, decant supernatant and use glass pipette to transfer sediment to InPouch TV. Place label on the spec info area of the pouch and place a large label on the Trich culture log.	
Ova and Parasites OP	For <i>Schistosoma haematobium</i>	15 mL of fresh urine. Centrifuge at 500 x g for 5-10 min. Decant supernatant and examine a drop of sediment microscopically.	


Department of Microbiology
Stool Specimen Processing Procedures



Test Name & Order Code	Specimen Types & Comments	Media	Incubation
Culture Stool w/Shiga Toxin CSTLST	Fresh or stool submitted in Cary-Blair enteric transport medium (not O&P preservative). If <i>Vibrio</i> , <i>Aeromonas</i> , or <i>Plesiomonas</i> requested, order routine culture and add indicated media.	CHROM Salm. (date/time) MAC CHROM O157 GN Broth (lid loose) CVA	Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C Microaerophilic 42 °C
Culture Stool w/ <i>Yersinia</i> & Shiga Toxin CSTLYS	Gram Stain (GSSM) may be ordered separately when requested.	TCBS (<i>Vibrio</i>)	Ambient at 35 ± 2 °C
Culture <i>Campylobacter</i> Screen CCAM		BAP (<i>Aeromonas</i>)	Ambient at 35 ± 2 °C
Culture <i>E. coli</i> O157 w/Shiga Toxin CECST		Add CIN to above set-up	Ambient at 30 °C
Culture <i>Yersinia</i> Screen CYER		CVA	Microaerophilic 42 °C
		CHROM O157 GN Broth (lid loose)	Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C
		CIN	Ambient at 30 °C
Test Name & Order Code	Specimen Types	Processing	
C difficile by PCR CDTPCR	Fresh	Label specimen and store in marked bin in the refrigerator. Place another label on the C diff PCR batch log.	
Ova and Parasites OP	Fresh Stool	Smear	Prepare smear and place directly into DMSO for 2 min, then ETOH 2 min, then to Trichrome stain jar.
		Conc.	Place stool in vial w/formalin to fix 30 min prior to conc.
	Unifix	Smear	Prepare smear and allow to air dry. Begin staining in Trichrome stain jar.
		Conc.	Use stool directly from Unifix vial to begin conc.
Fluorescent Parasite Smear CRYSM <i>Cryptosporidium/Cyclospora/Isospora</i>	Fresh, Unifix or Formalin	Process stool specimen following same concentration procedure as O & P. Use sediment to prepare smear and allow to air dry.	
Pinworm Preparation PIN	Scotch tape or Pinworm paddle	Place accession label on specimen and leave by O&P microscope for tech to examine.	

Department of Microbiology
Stool Specimen Processing Procedures




Test Name & Order Code	Specimen Types	Processing
Fecal Fat Qualitative FAT	Fresh	Label container and place specimen in marked bin in the refrigerator until someone is available to perform testing.
Stool Reducing Substances SRS	Fresh	Label container and place specimen in marked bin in the refrigerator until someone is available to perform testing.
Stool pH STPH	Fresh	Label container and place specimen in marked bin in the refrigerator until someone is available to perform testing.
Occult Blood OBLDSH 	Fresh	Apply thin smear of specimen inside Box A & B and close cover flap. Wait 3 - 5 min. Open perforated window in back of slide and add 2 drops of developer to each smear. Interpret within 60 s.





Department of Microbiology
Genital Specimen Processing Procedures

Test Name & Order Code	Specimen Types & Comments	Media & Direct Smear	Incubation
Culture Genital CGEN	Penis, cervix, vagina, urethra For endometrial or genital "wound" specs see CWD	BAP & MTM/CHOC Cervix/vagina: Carrot Broth (date & time) Gram Stain	CO ₂ at 35 ± 2 °C Ambient at 35 ± 2 °C
Culture GC Screen CGC	Vagina, cervix, urethra, throat, rectal	MTM/CHOC Gram Stain – not for throat or rectal	CO ₂ at 35 ± 2 °C
Culture Beta Strep B Screen CBSBS	Vaginal/Rectal	Carrot Broth (label with date & time) GBS Detect sub from negative Carrot Broth at 18-24 h	Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C
Group B Strep by PCR BSBPCR	Vaginal/Rectal	Lim Broth (label with date & time)	Ambient at 35 ± 2 °C For 12-18 h
Culture Yeast CYEST	Vaginal	CHROMagar <i>Candida</i> (date plate) Gram Stain	Ambient at 35 ± 2 °C (incubate in the dark)
IUD Culture CWD	IUD	BAP MTM/CHOC BAP Gram Stain if discharge on IUD	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Culture Placenta CTIS	Verify whether placenta was obtained via C-section or vaginal delivery.	C-section: see tissue culture set up Vaginal: credit Ana & Gram stain Inoculate BAP and Carrot Broth (label with date & time)	CO ₂ at 35 ± 2 °C Ambient at 35 ± 2 °C
Culture Semen CFL	Note: samples from NW Andrology & Cryobank, NW Cryobank, or NW Andrology are ordered and processed as CGEN w/out a smear	BAP MTM/CHOC split plate BAP + K disk Gram Stain Only add MAC if GNRs are seen in smear. CNA may be added if mixed organisms are seen in smear.	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C CO ₂ at 35 ± 2 °C
Culture to r/o <i>Haemophilus ducreyi</i> CWD	Swab from genital ulcer (chancroid)	Set up as CWD with a CHOC plate + VA disk (30 µg)	Taped, CO ₂ at 35 ± 2 °C



Department of Microbiology
Genital Specimen Processing Procedures

Test Name & Order Code	Specimen Types & Comments		Media & Inoculation	Incubation
Culture Trichomonas CTRICH PAML must submit specs. in the InPouch TV device. SH specs. may be submitted to Micro for inoculation. Process immediately!	Female	Vaginal	Place label in patient info area of pouch. Place pouch upright in a cup for 15 min to allow contents to settle before initial reading. For inpatient specimens not submitted in the InPouch TV device, refer to procedure for specific processing instructions.	Ambient at 35 ± 2 °C
	Male	Urethra Semen Urine		
Wet Mount  WMSH	Vagina or Cervix		Label specimen, slide, and a sterile, empty tube. Give to tech for testing.	
Vaginal Pathogens by DNA Probe VPDNAP or VAGPAN	Vaginal swab in Ambient Temperature Transport System		Label specimen and VPDNA log. Place specimen in holding rack.	
Culture Genital Mycoplasma / Ureaplasma CURMY Note: This test only detects <i>Mycoplasma hominis</i> and <i>Ureaplasma urealyticum</i> . Lower respiratory or lung specimens from adults are not appropriate for <i>M. hominis</i> and should be sent out for <i>M. pneumoniae</i> .	Urethral or cervical swab in M4 or M6 transport medium (refrigerated)		Vortex the tube. Inoculate an A7 agar by dipping a swab into the specimen and streaking the entire plate 3 times. Use a sterile transfer pipette to inoculate the 10B vial with 2-3 drops of transport media.	<u>A7 agar</u> Taped, CO ₂ at 35 ± 2 °C <u>10B broth</u> Ambient at 35 ± 2 °C
	Urine or body fluid (>2mL) submitted frozen OR Urine, semen, or body fluid or, for neonates, CSF, tracheal, or NP aspirate submitted in M4 or M6 transport medium (refrigerated).		Thaw frozen specimens. Centrifuge at 600 x g for 15 min. Remove supernatant, and use a drop of the sediment to inoculate the 10B. Use a sterile swab to streak a lawn on the A7 agar	
	Tissue		Mince tissue with sterile scalpel prior to inoculating media.	



Department of Microbiology
Wound and Body Fluid Specimen Processing Procedure

Test Name & Order Code	Processing	Media & Direct Smear	Incubation
Culture Wound CWD 	Ideally collected by aspiration. For specimens received on swabs, press and roll the swab to express absorbed material. Apply specimen in the center of the glass slide. For tissue samples see CTIS	BAP CNA MAC add CHOC for surgical specs BAP + K disk Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Culture Wound – Genital CWD  (Penis, cervix, vagina endometrium, urethra)	For routine genital culture (CGEN) or requests to r/o <i>Haemophilus ducreyi</i> , see Genital Specimen Processing Procedures above.	Same as CWD plus MTM/CHOC	CO ₂ at 35 ± 2 °C
Culture Body Fluid CCSF  <ul style="list-style-type: none"> • Cerebral spinal fluid (CSF) • Ventricular fluid • Subdural fluid <p>Always STAT!</p>	<p>Clear fluids that are > 1 mL:</p> <ol style="list-style-type: none"> 1. Prepare smear by cytocentrifuge 2. For Cx, centrifuge 15 min at 3,000 x g 3. Transfer supernatant into sterile tube labeled with accession label 4. Use sediment to inoculate media <p>Clear fluids that are ≤ 1 mL:</p> <ol style="list-style-type: none"> 1. Prepare smear by cytocentrifuge 2. Use fluid for direct culture inoculation <p>Grossly bloody fluids:</p> <ol style="list-style-type: none"> 1. Do not centrifuge 2. Use fluid directly for inoculation of culture and smear <p>Turbid fluids:</p> <ol style="list-style-type: none"> 1. Use fluid directly for smear preparation 2. For Cx, centrifuge 15 min at 3,000 x g 3. Pour supernatant into sterile tube labeled with accession label <p>Use sediment to inoculate media</p>	BAP CHOC Gram Stain MAC if GNRs are seen in smear  BAP Ana if bacteria are seen in the smear.	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C


Department of Microbiology
Wound and Body Fluid Specimen Processing Procedure

Test Name & Order Code	Processing	Media & Direct Smear	Incubation
<p>Culture Body Fluid - CFL <u>Joint fluid (synovial)</u></p> <ul style="list-style-type: none"> • Knee fluid • Elbow fluid • Shoulder fluid • Ankle fluid • Finger fluid <p><u>Other effusions</u></p> <ul style="list-style-type: none"> • Amniotic • Culdocentesis • Prostatic • Pleural/Thoracentesis • Other body fluids 	<p>Clear body fluids that are > 1 mL:</p> <ol style="list-style-type: none"> 1. Centrifuge 15 min at 3,000 x g. 2. Transfer supernatant into sterile, labeled tube. 3. Resuspend sediment. 4. Use sediment for smear prep and media inoculation. <p>Clear fluids that are ≤ 1 mL: Use fluid directly.</p> <p>Grossly bloody fluids: Use fluid directly.</p> <p>Turbid fluids:</p> <ol style="list-style-type: none"> 1. Use fluid directly for smear preparation. 2. For culture, centrifuge 15 min at 3,000 x g. 3. Transfer supernatant into sterile tube labeled with accession label. 4. Use sediment to inoculate media. <p>Clotted fluids: </p> <p>Transfer clotted material to a Whirl-Pak and process as described under CTIS to release any trapped bacteria.</p>	<p>BAP CHOC BAP + K disk Gram Stain</p> <p>Only add MAC if GNRs are seen in smear. CNA may be added if mixed organisms are seen in smear.</p>	<p>CO₂ at 35 ± 2 °C CO₂ at 35 ± 2 °C Ana at 35 ± 2 °C</p> <p>CO₂ at 35 ± 2 °C</p>
<p>Culture Body Fluid - CFL <u>Abdominal effusions</u></p> <ul style="list-style-type: none"> • Abdominal • Ascitic • Paracentesis • Peritoneal • Pelvic Fluid 	<p>When accessioning, note "blood bottles added" unless ≤ 1 mL is received.</p> <p>Clear fluids:</p> <ol style="list-style-type: none"> 1. If ≥ 70 mL, centrifuge a 50 mL aliquot at 3,000 x g for 15 min. Use the sediment for smear prep and media inoculation. Inoculate extra, non-centrifuged fluid into AER & ANA bottles (maximum 10 mL in each). 2. For smaller volumes (> 1 mL), centrifuge specimen and aseptically transfer supernatant to a separate sterile container. Use the sediment for smear prep and media inoculation. If the entire specimen was centrifuged, resuspend the pellet with supernatant and use for inoculating AER & ANA bottles. 3. If ≤ 1 mL, use specimen directly for smear prep and media inoculation. Do not add AER/ANA bottles. <p>Grossly bloody or turbid fluids:</p> <ol style="list-style-type: none"> 1. Use directly for smear, plates, and AER & ANA. <p>Clotted fluids: </p> <p>Transfer clotted material to a Whirl-Pak and process as described under CTIS to release any trapped bacteria.</p>	<p>BAP CHOC BAP + K disk Gram Stain</p> <p>Only add MAC if GNRs are seen in smear. CNA may be added if mixed organisms are seen in smear.</p>	<p>CO₂ at 35 ± 2 °C CO₂ at 35 ± 2 °C Ana at 35 ± 2 °C</p> <p>CO₂ at 35 ± 2 °C</p>

Department of Microbiology
Wound and Body Fluid Specimen Processing Procedure

Test Name & Order Code	Processing	Media & Direct Smear	Incubation
Culture Body Fluid – CFL Bone Marrow 	Label original tube, smear, and media. Leave the rest of the labels with the media at room temperature to be used for ISOLATOR processing.	BAP CHOC BAP + K disk No Smear (credit)	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Colony Count Dialysis Water or Dialysate CCDW or CCDI	Pipette 500 µL and spread over agar surface.	BAP	Ambient at 35 ± 2 °C
Culture Donor Milk CBMLK 	Pipette 100 µL and spread over agar surface.	BAP	Ambient at 35 ± 2 °C

**Department of Microbiology
Tissue Specimen Processing Procedure**

Test Name & Order Code	Specimen Types & Comments	Media & Direct Smear	Incubation
Culture Tissue CTIS	If tissue is more than 1 cm ³ , use sterile forceps and scalpel to cut into sections. Use cut tissue to make touch preps for smears. Then process tissue using the Whirl-Pak. Place tissue in a Whirl-Pak bag with 2 mL of sterile saline. Expel as much air as possible and seal bag. Place inside of a second Whirl-Pak. Roll a thick marking pen over bag until tissue is dispersed in the saline. Use a sterile pipette to inoculate the homogenized material to appropriate media. Save the specimen in the bag, along with leftover tissue, at -70°C.	BAP CHOC CNA MAC BAP + K disk Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Culture Tissue (<i>H. pylori</i>) CHP  For any tissue specimens that are labeled gastric, antrum, esophagus, fundus, or duodenum.	For gram stain, make impression smears BEFORE grinding the tissue. Homogenize the specimen in Whirl-Pak with 0.5 mL of sterile saline and use to inoculate BAP. Place sign on incubator and a note on the jar with a preliminary date at day 3 and final at day 7.	BAP Gram Stain	Microaerophilic at 35 ± 2 °C
<i>Helicobacter pylori</i> Screen (CLO Test) HPS	Verify collection date if not clearly indicated. Place label on the CLO test device and on the log sheet with the receipt time. Incubate for 3 h. Examine the test for color change from yellow to magenta pink. If positive reaction is noted, record on the log and enter result in LIS. After 3 h, if the test is negative, the set-up bench person will remove the test from the incubator, and leave at room temperature by the O & P microscope to be reported on the following day. Record the 24 h result on the log sheet and enter result in LIS.	CLO Test	Ambient at 35 ± 2 °C

Department of Microbiology
Upper Respiratory Specimen Processing Procedure





Test Name & Order Code	Specimen Types & Comments	Media & Direct Smear	Incubation
Culture Beta Strep A Screen CBSAS	Throat	BAP + A disk	Ana at 35 ± 2 °C
Culture GC Screen CGC	Throat - Do not prepare a Gram stain	MTM/CHOC No GS	CO ₂ at 35 ± 2 °C
Culture Respiratory: CF CRCF	Throat	BAP CHOC MAC MSA BSA Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C
Culture Sinus CWD	See wound set-up. Include CHOC plate.		
Culture MRSA Screen CMRSA		CHROM MRSA II Label with date & time	Ambient at 35 ± 2 °C
MRSA Nasal Screen by PCR MRSPCR	Nasal swab in Amies Gel or Liquid Stuart transport medium. Label specimen and MRSA PCR log. Place specimen in holding rack in refrigerator.		
Culture Bordetella pertussis CBPERT	NP swab in Amies Gel w/Charcoal. If left and right NP swabs are submitted, use both swabs for one culture. or NP washing/aspirate in sterile container.	Regan Lowe (seal w/tape)	Ambient at 35 ± 2 °C
Pertussis FA Stain PERTSM	Slides with NP secretions. Label slides and place on warmer by Blood bench.		
Request for diphtheria culture	Send out to ARUP or WA DOH		

Department of Microbiology
Lower Respiratory Specimen Processing Procedure



Test Name & Order Code	Specimen Types & Comments	Media & Direct Smear	Incubation
Culture Lower Respiratory CRESP	Sputum, Bronchial washings, BAL. Bronchial brush (quantitative) should be received in 1 mL of Ringer's lactate. Vortex prior to sampling. Inoculate plates and smear using 0.01 mL calibrated loop. Note: If Gram stain suggests <i>S. pneumoniae</i> :	BAP CHOC MAC Gram Stain BAP + P disk	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Culture Respiratory: CF CRCF	Sputum	BAP CHOC MAC MSA BSA Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ambient at 35 ± 2 °C Ambient at 35 ± 2 °C
Culture AFB (Mycobacterium) CAFB	See Culture AFB set-up		
Culture Fungus CFC	See Culture Fungus set-up		
Culture <i>Legionella</i> CLEG	Bronchial washings, Sputum, Pleural, Lung tissue	BCYE BCYES (seal w/tape)	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C
<i>Legionella</i> FA Stain LEGSM	Sputum or Bronchial washings	Mucolyse if necessary and cytospin	
	Pleural fluid	Centrifuge larger volumes and use sediment for smear preparation. Cytospin smaller volumes.	
	Lung tissue	Cut with sterile scalpel and make touch preps	
<i>Pneumocystis</i> FA Stain PNESM	Sputum or Bronchial washings	Mucolyse if necessary and cytospin	
Ova and Parasites OP	Sputum or Bronchial washings	Don't concentrate. Perform direct wet mount for larvae and ova.	

Department of Microbiology
Eye and Ear Specimen Processing Procedure

Test Name & Order Code	Specimen Types & Comments	Media & Direct Smear	Incubation
Culture Eye CEYE	Conjunctiva swab	BAP CHOC	CO ₂ at 35 ± 2 °C
Culture Fluid CFL	Vitreous fluid	BAP	CO ₂ at 35 ± 2 °C
Culture Wound CWD 	Corneal scraping	Gram Stain	Ana at 35 ± 2 °C
Culture Wound (cornea donor) CWD 	Do not set up a thio on swab corneal specimens.	BAP CHOC BAP Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C
Culture Tissue (corneal donor) CTIS	When corneal tissue is received in thio broth, aseptically transfer small amount of thio to plates. Place thio tube in incubator rack. If corneal tissue is received in saline, aseptically transfer small amount of saline to plates. Using sterile forceps, aseptically transfer corneal ring into thio tube and place incubator.	BAP CHOC BAP Thio Broth Credit Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C Ambient at 35 ± 2 °C
Culture Ear CEAR		BAP CHOC CNA MAC BAP + K disk Gram Stain	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C

Department of Microbiology
Blood Culture and IV Catheter Specimen Processing Procedure



Test Name & Order Code	Comments	Media	Incubation
Culture IV Catheter Tip CCATH	Aseptically transfer catheter tip to BAP. If the tip is too long to fit on the BAP, cut it in half with a sterile scalpel and use both pieces for culture. Roll tip back and forth over agar surface four times in four different directions.	BAP	CO ₂ at 35 ± 2 °C
Culture Blood CBLD	One set consists of an aerobic bottle and an anaerobic bottle. Only one set of bottles can be placed under an accession number. Blood may be submitted in SPS tubes for specific pathogens (e.g. <i>Bartonella</i> , <i>Francisella</i> , <i>Brucella</i>). Process using Isolator method. If non-Bact/ALERT bottles are received, refer to Odd Bottle Procedure.	Bact/ALERT	Bact/ALERT
Culture Blood Fungus CBF	See Fungus Culture Processing Procedure		
Culture Blood Component (Blood Bank) CFL	Using sterile technique, withdraw 10 mL of the blood component from the bag and inoculate an aerobic blood culture bottle. Change source in blood computer. Unless requested, credit Gram stain in LIS. Enter result, "Gram stain credited. Specimen in blood bottle only."	Aerobic blood culture bottle	Bact/ALERT

Department of Microbiology
Drug Resistant Organism Specimen Processing Procedure



Test Name & Order Code	Comments	Media & Direct Smear	Incubation
Culture ESBL Screen CESBLS	Inoculate media and incubate according to specimen source.		
Culture MRSA Screen CMRSA		CHROMagar MRSA II Label with date & time	Ambient at 35 ± 2 °C
Culture VRE Screen CVRE		LKV	Ambient at 35 ± 2 °C
MRSA Nasal Screen by PCR MRSPCR	Nasal swab in Amies Gel or Liquid Stuart transport medium. Label specimen and MRSA PCR log. Place specimen in holding rack in refrigerator.		

Department of Microbiology
Fungus Culture Specimen Processing Procedure


Test Name & Order Code	Specimen	Processing	Microscopy	Media	Incubation
Culture Blood Fungus CBF	Blood should be submitted in SPS or Isolator tubes for dimorphic fungi (typically <i>Histoplasma</i>). Process specimen using Isolator method and inoculate routine fungus media. If Bact/ALERT bottles are received from an inpatient, call to clarify order. If clinician wants to r/o dimorphic fungi, blood should be recollected in a SPS or Isolator tube. If yeast is suspected, order should be changed to CBLD. For PAML clients, initiate a CRM case to clarify order.		Not performed	SDA BHIA	Ambient at 30 °C Ambient at 30 °C
	Bone marrow specimens must be submitted in either SPS or ISOLATOR tube.	Label original tube, smear, and media. Inoculate smear directly. Leave the rest of the labels with the media at room temperature to be used for ISOLATOR processing.	Calcofluor		
Culture Fungus CFC	CSF	<1 mL, inoculate directly >1 mL, centrifuge 15 min at 3,000 x g. Remove and save supernatant. Use sediment to inoculate media & smear.	Calcofluor	SDA BHIA CHROM <i>Candida</i>	Ambient at 35 ± 2 °C (date CHROM and incubate in dark)
	Body Fluids	Refer to processing instructions for CFL prior to inoculating media and smear.	Calcofluor		
	Tissue	Place tissue in sterile petri dish. Use sterile scalpel to cut into sections. Place a thin piece of tissue on each piece of media on the center of the plate. Grind remaining tissue in Whirl-Pak and inoculate media with 3 diagonal streaks.	Calcofluor (touch prep)		
	Sputum	Use swab to select mucoid or bloody portions to inoculate media & smear.	Calcofluor		
	Bronch wash/ Lung aspirate	Inoculate media and smear directly	Calcofluor		
Fungus Stain FSM	Any of the above	See above	Calcofluor		

Department of Microbiology
Fungus Culture Specimen Processing Procedure

Culture Fungus Skin, Hair, Nails CFS	Skin	Inoculate media by pressing small pieces of skin scrapings into the agar.	KOH (time) w/Calcofluor	SDA BHIA	Ambient at 30 °C Ambient at 30 °C
	Hair	Use sterile tools, cut hair into small pieces. Press a few pieces of hair into the agar of each plate.	KOH (time) w/Calcofluor		
	Nails	If whole nail received, scrape with sterile scalpel into a petri dish. Inoculate media by pressing small pieces of nail into agar.	KOH (time) w/Calcofluor	SDA BHIA	Ambient at 30 °C Ambient at 30 °C
Culture Yeast CYEST	Throat, Vagina, Stool, Urine	Inoculate and streak for isolation. For urine specs, refer to urine processing for quantitative inoculation.	Gram Stain	CHROM <i>Candida</i> (date)	Ambient at 35 ± 2 °C (incubate in the dark)

Department of Microbiology
AFB Culture Specimen Processing Procedure



Test Name & Order Code	Specimen	Processing & Set-up
Culture AFB CAFB	Sputum, bronchial washings, BAL, or any specimen in which it would be important to decontaminate and/or break down mucous should be digested.	Label slide and etch for smear. Label 1 LJ slant and place extra label on lid to be used for the MGIT vial. Label 50-mL sterile conical centrifuge tube. Pour approximately 5 mL of specimen into tube. Place specimen in refrigerator to be decontaminated with next batch.
	Skin or subcutaneous soft tissue, including punch biopsy, external abscess, wound, cornea  (not for inpatient surgical specimens) Note: extra media are used for these body sites. Ask supervisor, director, or technical specialist if unsure about adding extra media. If no one is available to ask, add the extra media.	Swab: Label slide and etch for smear. Label 2 LJ slants and 1 CHOC plate. Place extra label on 1 of the LJ lids to be used for the MGIT vial. Label 50-mL sterile conical centrifuge tube. Add 2-3 mL of sterile saline. Insert swab in tube and leave in rack for decontamination. Skin/subcutaneous tissue biopsy: Label slide and etch for smear. Label 2 LJ slants and 1 CHOC plate. Place extra label on 1 of the LJ lids to be used for the MGIT vial. Homogenize tissue in Whirl-pack bags with 2 mL of sterile saline. Inoculate media if routine or fungal testing is ordered. Transfer remaining fluid to a labeled 50-mL conical centrifuge tube and place in refrigerator for decontamination with next batch.
	Any specimen from a <u>normally sterile</u> body site (e.g. CSF, pleural fluid, joint fluid, etc) Note: These specimens should be inoculated to LJ and MGIT media directly, without decontamination/digestion. For lymph node specimens, add CHOC and extra LJ as described above for subcutaneous tissue.	Label slide and etch for smear. Label 1 LJ slant and place extra label on lid to be used for the MGIT vial. Fluids: if ≤ 1 mL, mark "direct" on original tube and place in refrigerator. For volumes > 1 mL, centrifuge up to 50 mL at 3,000 x g for 15 min. Using a sterile pipette, remove all but 1 mL of the supernatant. Use sediment to inoculate routine or fungal media if requested. Mark "direct" on tube and place in refrigerator with next batch of AFB specimens. Tissues: homogenize tissue in Whirl-pack bags with 2 mL of sterile saline. Inoculate media if routine or fungal testing is ordered. Transfer remaining fluid to a labeled 50-mL conical centrifuge tube. Mark the tube with "direct" and place in refrigerator with next batch of AFB specimens.

Department of Microbiology
AFB Culture Specimen Processing Procedure



Test Name & Order Code	Specimen	Processing & Set-up
Culture AFB CAFB	Stool	Label slide and etch for smear. Label 1 LJ slant and place extra label on lid to be used for the MGIT vial. In a sterile, 50-mL conical centrifuge tube, emulsify about 1 g of stool in 2-3 mL of sterile saline. If liquid, transfer 2-3 mL of stool to the tube. Place labeled specimen in refrigerator to be decontaminated with next batch.
	Gastric	Label slide and etch for smear. Label 1 LJ slant and place extra label on lid to be used for the MGIT vial. Transfer specimen to a 50-mL conical centrifuge tube. Place labeled specimen in refrigerator to be decontaminated with next batch. If > 5 mL of specimen is received, centrifuge up to 50 mL at 3,000 x g for 15 min. Using a sterile pipette, remove all but 5 mL of the supernatant and then leave specimen in the refrigerator to be decontaminated with the next batch. Note: specimens that will be in transport more than 4 h should be neutralized with 100 mg sodium carbonate. Conical tubes with sodium carbonate are available in the set-up area to send to clients.
	Urine	Label slide and etch for smear. Label 1 LJ slant and place extra label on lid to be used for the MGIT vial. Centrifuge up to 50 mL at 3,000 x g for 15 min. Using a sterile pipette, remove all but 5 mL of the supernatant. Place specimen in refrigerator to be decontaminated with next batch.
Culture, AFB (No Smear) CAFBNS	Blood or bone marrow: Specimens must be submitted in either SPS or ISOLATOR tube. Alternatively, bone marrow < 5 mL may be submitted in heparin.	Label original collection tube and 1 LJ tube. Leave the rest of the labels with the tube to be used for ISOLATOR processing and for labeling the MGIT tube. Place specimen at room temperature with LJ tubes for next batch.

Department of Microbiology
Organism Identification Specimen Processing Procedure

For bacterial and yeast isolates submitted for ID and/or AST:

Label slant or plate received with accession label. Label subs with accession label and date & time. **Perform smear preparation for Gram stain and culture subbing in biosafety cabinet.** Place subs in appropriate incubators and leave any paperwork on assigned bench.



Test Name & Order Code	Sub to Media	Incubation	Assigned Bench
ID Organism w/Susceptibility CIDS ID Organism CORG	BAP & CHOC MAC (if GNR) BAP	CO ₂ at 35 ± 2 °C CO ₂ at 35 ± 2 °C Ana at 35 ± 2 °C	Wound/Respiratory New Bench or Blood Bench (depending on source)
	CHROMagar Salm. (if Salmonella requested)	Ambient at 35 ± 2 °C	5 th New Bench
	CHOMagar O157 (if E. coli O157 requested)	Ambient at 35 ± 2 °C	5 th New Bench
	CVA (if Campy request)	Microaerophilic 42 °C	5 th New Bench
Susceptibility Test SUSC	BAP & CHOC	CO ₂ at 35 ± 2 °C	Wound/Respiratory New Bench or Blood Bench (depending on source)
ID Organism Urine CORGUR ID Organism Urine w/Susc. CURIDS	BAP	Ambient at 35 ± 2 °C	Urine New Bench
	MAC (if GNR)	Ambient at 35 ± 2 °C	
ID AFB AFBID	No subbing in set-ups. Performed technical staff in Mycobacteriology.		Mycobacteriology (AFB)
ID Fungus (Mold) FUNGID	No subbing in set-ups. Performed technical staff in Mycology.		Mycology (Fungus)
ID Yeast YID	CHROMagar Candida Sabouraud Dextrose	Ambient at 35 ± 2 °C (incubate in dark)	Mycology (Fungus)
Parasite Identification, Macroscopic PARID	Place accession label on specimen. Leave by O&P microscope for tech to examine.		