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Introduction

Specimen processing is an essential for accurate testing results. Many microbiological tests rely heavily on specified processing instructions and is the most important step in the recovery of pathogenic organisms. Treatment and handling of any specimen must be done in a safe and ethical manner following the procedures described below. Specimens are to be used tested only as requested and according to laboratory protocols. Proper approval and consent must be received for samples to be used for any other purpose

Secondary Specimen Labelling

Adequate specimen information must be provided on any secondary items related to a sample throughout all phases of testing. Secondary items include aliquots, tubes, slides, culture plates, extraction vessels, images etc created during testing.

- A single **unique** identifier (e.g. specimen number - LIS or auxiliary number) is sufficient to labels materials derived from the primary specimen for use in testing and must be able to be linked to the full patient and specimen specific information. This includes barcodes.
- The identifier must be legible and clear throughout all processing and conditions of storage.

General Processing Instructions:

- Print required labels one sample at a time, removing all labels from the printer before proceeding with subsequent samples.

- Process one specimen in the Biological Safety Cabinet (BSC).
- Refer to the Laboratory Safety Manual for best working practices in a BSC.

- Change gloves and perform hand hygiene after manipulating biological samples.

- Follow specific processing procedures per testing method used as described in each testing manual. (ie. PCR, Serology etc)

Aliquoting

Aliquoting or transferring primary specimens may be necessary for the testing process. Aliquots are considered a secondary specimen container and must follow secondary specimen labeling requirements.

Potential risk exists for errors when aliquoting including cross contamination.

When aliquoting:

- Work with one specimen at a time.
- Compare sample identifiers of primary and secondary containers.
- Use a new pipette for each sample.

Never transfer an aliquot back into its original container.

Planting Specimens in ESwabs

Planting by WASP

Use the WASP routinely to plate all specimens collected with ESwab.
WASP will inoculate 30µL of the ESwab transport medium to each media plate.
For WASP operation instructions, please see

Manual Planting

For ESwabs with fluid:

1. Votex the ESwab.
2. Use a transfer pipette and inoculate 1drop of ESwab transport medium onto each media.

*For ESwabs with **NO** fluid:*

1. Use swab to inoculate each media
2. In the culture "Test Comment" add comment **}NFLD** found in the rejection keypad

Broth Inoculation for Infection Control Outbreak Investigation

1. Place 500uL (0.5 mL mark of transfer pipette) of the ESwab transport medium into a 3 mL tube of Brain Heart Infusion broth (BHIB), incubate overnight on the shaker at 37^{o}C in the walin incubator.
2. Place 30uL (1 drop from transfer pipette) of the ESwab transport medium onto Specific Screening Agar, incubate agar as per routine screening protocol.
3. After overnight incubation, subculture the BHIB onto Specific Screening Agar, incubate agar as per routine screening protocol.

BLOOD CULTURE

Operation of the Virtuo System :

Blood culture Bottles are tracked by the Virtuo system and communicate directly with the LIS. All information about the Virtuo and BC bottles can be accessed on the large or small display screen.

The Virtuo manual is located beside the system for reference.

The large display screen
This screen monitors a number of functions including positive and negative bottles, warnings and errors, and is used to generate reports, searches and edit bottle data. These are controlled through 5 icons:

1. Home: Select this icon anytime to display the main large display screen.

2. Search: Use this icon to search for an order/ bottle to view or edit any related information (Order number, incubation time etc)

3. Display Bank: Select this icon to display information specific to each connected unit.

4. Reports: Select this icon to generate different reports.

5. Configure: Select this icon to view Virtuo configuration.

The small display screen
The small display screen is located above the bottle return chute and display 2 icons.

1. Unit temperature: Displays unit temperature in degrees Celsius.

2. Waste level: Displays waste level used in percentage.

Accessioning BC Bottles:

i. For Isolator tubes follow Isolator 10 protocol
ii. For C&S order on BC bottles or Fluids received in BC bottles refer to section: Fluids received in BC bottles
iii. Enter any specimen comments or BCs with **special incubation times** (see table below) in "Order Comment" in the LIS

Blood Culture	**5 days**	**7 days**	**14 days**	**21 days**
Routine (Blood, Bone marrow, Sterile fluids, Blood products)	X			
Fungus/Yeast	X			
Dimorphic Fungi & Cryptococcus* (Histoplasma, Blastomyces) **notify ward / physician must also collect Isolator collection tube if not received. Process BC Bottles as per normal protocol*	X			
Bone Bank Blood		X		
ENACT Sterilities			X	
Brucella				X
PD Effluent				X
SBE/IE, PUO/FUO (Subacute bacterial endocarditis/Infective endocarditis & Pyrexia of unknown origin/Fever of Unknown origin				X

iv. Place barcodes vertically without covering the bottle barcode.

Loading Bottles:

DO NOT load macroscopically positive bottles (yellow septum). Follow protocol for processing positive BC bottles

i. Ensure each set of BC bottles are matching (same LIS/aux #). If mismatched bottles arrive, wait for other set or phone ward to correct issue.
ii. Ensure labels are affixed vertically and bottle barcode is visible. If bottle barcode is covered, see Bottles with Unreadable or Generic Labels

iii. Clean bottles with 3% hydrogen peroxide or Virox wipes

iv. Place bottles upright on the conveyor belt which automatically load into the Virtuo.
DO NOT LOAD MORE THAN 40 BOTTLES AT A TIME
- Before walking away, verify the instrument is operating properly and bottles remain upright and are being properly loaded into the bottle indexer. Monitor instrument for any flashing indicatory lights.

For bottles with unreadable or generic labels:

If a bottle barcode or LIS label is unreadable/covered or a generic label was used it will be flagged as anonymous bottle and an alert will appear.

i. Log in using your user name and password
ii. Select the anonymous bottle alert.
- If multiple anonymous bottles exist, they appear one record at a time for editing

iii. If visible from the bottle image, enter bottle ID and/or type and save record.
iv. If the bottle ID cannot be seen / generic label not applied, unload bottle.
- An alert indicating a label is required will be displayed
- Place a generic label on the bottle and reload bottle.
- You must specify the bottle type in Virtuo

Unloading Negative Bottles:

- Negative bottles automatically unload and drop into the garbage container.
- Dump garbage into double-bagged, cardboard waste box. Label outside of the box with date and controller the bottles were removed from.
- A negative result is automatically released and finalized upon unloading.
- The waste capacity is noted on the top right corner of the small display.
  - An alert will display on the large display if the capacity is full or almost full.

Processing macroscopically positive bottles not loaded in Virtuo:

Macroscopically positive bottles have a lemon-yellow sensor disc or a bulging septum.

- Process **ONLY** the suspected positive bottle (do not sub-culture the matching bottle unless it is also macroscopically positive or flagged positive).

- Follow the appropriate positive subculturing procedure for the positive bottle.
- Do not load macroscopically positive bottles into the Virtuo. They may not be flagged as positive and result in false negative results.

Processing BC Bottles Flagged Positive:

Positive bottles will alert on the Virtuo and show a count of positive bottles on the screen.

i. Touch "+" sign to unload positive bottles to the bottle retrieval area.
ii. Only one bottle can unload to each chute in the retrieval area at one time. Remove these in order for further positive bottles to be unloaded.
iii. Write controller number on the bottle when you remove it.
    - Bottles which need to be reloaded, must be returned to the controller where they originated.
iv. When bottle is removed the positive bottle count updates and the unload event is recorded.

*Note: If a culture bottle marked as "**Brucella**" is flagged positive, remove a small amount of the culture for a Gram smear ONLY. Notify technologist. DO NOT SUBCULTURE until further instructions from technologist.*

*Note: If a culture bottle is marked with ESO flag "**VDE alert for micro lab**", please include subculture to BVRE in addition to routine culture media to aid in isolation of vancomycin dependent enterococci..*

Subculturing: bottles for WASP Streaking

i. In softmic open **result entry**
ii. Scan the bottle LIS barcode
iii. Go to the media section, the appropriate bottle (FO2V, FNV or PEDV)
iv. From the keypad, pick **1. {BC+**
    - If there is already an anaerobic bottle subbed, sub the bottle manually.
v. Click the Order/Entry icon
vi. Click "Yes" to save changes to Result entry
vii. From the order entry keypad, Click **Blood Culture** |>BLD
viii. Pick "**Aerobic BC subculture**" or "**Anaerobic BC subculture**" or "**Paediatric BC subculture**"
ix. Click the "Save" icon
x. Print **one** specimen label for subculture tube.
xi. Put the large barcode label, the FO2 or FN or PED bottle and one new red capped BC+ subculture tube into a cup in the rack.

xii. Placing only one cup in the hood at a time, affix the large barcode label to a red capped BC+ tube for the WASP
xiii. Clean the top of the blood culture bottle and the top of the BC+ tube with an alcohol swab.
- Inspect bottle septum. If obviously bulging, use a venting unit to safely offload the gas.

xiv. Place a needle adaptor into the blood culture bottle.
xv. Turn the bottle upside down (45degree angle), with the adaptor in place. Making sure the needle is submerged in the blood.
xvi. Allow the resin to settle (5-10 seconds)
xvii. Insert the BC+ tube on the other end of the needle adaptor; allow 1.5 mL to 2 mL of blood to transfer from the bottle into the tube. There is an indicator (red zone) on the BC+ tube label. Ensure the blood is within the red zone.
xviii. Place the blood culture in the numbered storage rack. Date the first positive bottle with today's date.
xix. Place the tube in a WASP palette. Load no more than 3 BC+ blood tubes per pallet.
- Run wash loop after every 3 BC+ tubes loaded

xx. Place wasp specimen rack on Wasp 3. It is programmed to subculture and do a gram stain.
xxi. Ensure that plates are streaked and put into appropriate incubators STAT
xxii. Stain gram smear as per Gram Stain protocol STAT
xxiii. Give the stained smear to the STAT technologist (or designate) STAT
xxiv. Store red-capped BC tube from the wasp in storage boxes (as e-swabs are saved) at room temperature.

Subculturing: if WASP rejects the BC+ tube or the second bottle

i. In softmic open **result entry**
ii. Scan the bottle LIS barcode
iii. Go to the media section, the appropriate bottle (FNV, FO2V, SAV/SNV, or PEDV)
iv. From the keypad, pick **1. {BC+**
v. Press the "Save' icon
vi. Print media labels
vii. From labels printed, on one small barcode label, write the bottle code of the positive bottle (e.g. FN/FA/P/SAV/SNV)
- Place one label on a slide for the gram stain with date i.e. FN6

viii. On the large Media labels, write the bottle code and date bottle turned positive (e.g. FN 21)
ix. Label media and sub-culture 2 drops onto the following (whole) plates:

Media		Incubation
Blood Agar (SUBBA)	CO_2	35°C x 48 hours
Chocolate Agar (SUBCH)	CO_2	35°C x 48 hours

MacConkey Agar (SUBMC)	CO_2	35°C x 48 hours
Fastidious Anaerobic Agar (SUBBR)	AnO_2	35°C x 48 hours

x. Inoculate slide with one drop of specimen for gram stain and spread drop with a clean slide.
- Place slide on heading block to dry.
- Stain as per Gram Stain protocol

xi. Put plates on ISOplater for streaking

xii. Place ANO_2 plates in ANO_2 holding jar.

xiii. Give stained slide to technologist

xiv. Place CO_2 plates in rack labeled "New Positive. No Maldi Yet" in CO2 incubator in planting area

xv. Keep bottles in the Positive bottles tray until the isolate has been frozen and the final report has been issued.

Manual Back-up Protocol

If the automated system should fail for >24 hours or if expired bottles, non-Virtuo bottles are received, the following procedures will be used.

1. Examine all bottles for macroscopic growth twice daily for day 1 and day 2, record results in the LIS.
2. Ensure BCBC media is checkmarked under column R, this prevents the sample from automatically finalizing.
3. For the remainder of the incubation period check bottles macroscopically at least daily and record in the LIS
4. For macroscopically positive bottles, process as indicated under "Positive Cultures".
5. If macroscopically negative, sub-culture as follows:

Direct Examination: Not required.

Sub-culture:

Incubation Day	Bottle Type	Medium	Incubation
1	Aerobic bottle	CHOC	CO_2, 35°C, x 48 hours
2 & 5	Aerobic and Anaerobic bottle	CHOC & BRUC	CO_2, 35°C, x 48 hours AnO_2, 35°C, x 48 hours

SPS Blood Culture Protocol for Dimorphic Fungi

Isolate 10 system collection tubes are currently unavailable. Follow this protocol utilizing SPS vacutainer tubes in lieu as a secondary protocol only to used until isolate tubes again become available.

Order Entry in Site Field: Add "**SPS TUBE**"

1. Centrifuge specimen at 3000g for 30 minutes

Working in a biosafety cabinet:

2. Clean vacutainer stopped and top of tube with virox or equivalent. Remove vacutainer stopper
3. Using an individually wrapped sterile pipette, remove and discard supernatant, not disrupting the buffy coat.
4. Place the original stop back onto the tube and vortex tube for at least 10 seconds on the highest setting.
5. Using a new individually wrapped sterile pipette, slowly aspirate concentrate left in tube.
6. Dispense concentrate in a straight line along the surface of the agar. Keep inoculum away from the edge of the plate

Media		Incubation
Inhibitory Mold Agar (IMA)	O_2,	28°C x 4 weeks
Esculin Base Medium (EBM)	O_2,	28°C x 4 weeks
Two Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)	O_2,	28°C x 4 weeks

7. Using the tip of the pipetting, streak the plate. Use 15-20 passes perpendicular to the original inoculum line.
8. Dry plates. Seal with parafilm and forward plates to Mycology for incubation and processing.

Handling of Body Fluids in Blood Culture Bottles

Order/Entry:

1. **PD effluent fluids arrive into microbiology in Blood Culture Bottles**

i. At Order/Entry Screen, call up order by Auxiliary number.
ii. Add the test "Fluid in BC Bottle" (?BTLE)
iii. Receive and plate as usual.
iv. Add bottles received under media for C&S test
v. Apply the specimen labels on the bottles (the labels with the lab number that contains the extension 93). **DO NOT** use the media labels or the specimen label for the C&S or FLDM test.
vi. Load the bottles to Virtuo as usual by scanning the bottle barcode and then the specimen barcode.
vii. Ensure the incubation time is changed 21 days for both bottles.

2. **Sterile Body fluids, Blood bank products and Bone Marrow put in a Blood Culture Bottle ONLY.**

i. At Order/Entry Screen, call up order by Auxiliary number.
ii. Add the test "Fluid in BC Bottle" (?BTLE)
iii. Receive and plate as usual.
iv. Before saving, to go to media screen. Cancel the media that are attached to the C&S or FLDM test and add appropriate bottle media codes (ex FO2, FN, ANAO2…)
v. Save the order and print the labels
vi. Apply the specimen labels on the bottles (the labels with the lab number that contains the extension 93). DO NOT use the media labels or the specimen label for the C&S or FLDM test.
vii. In result entry, result Gram as "Not Applicable" and finalize.
viii. Load the bottles to Virtuo as usual by scanning the bottle barcode and then the specimen barcode.
ix. These all have an incubation time of 5 days for the bottles.

3. Sterile Body fluids, Blood bank products and Bone Marrow that are sent to the lab in a sterile container AND a set of blood culture bottles.

i. At Order/Entry Screen, call up order by Auxiliary number.
ii. Add the test "Fluid in BC Bottle" (option 1)
iii. Receive and plate as usual.
iv. Do not cancel the media that are attached to the C&S / FLDM test as they will be processed. add appropriate bottle media codes (ex FO2, FN, ANAO2…)
v. Save the order and print the labels
vi. Apply the specimen labels on the bottles (the labels with the lab number that contains the extension 93). DO NOT use the media labels or the specimen label for the C&S or FLDM test.
vii. Load the bottles to Virtuo as usual by scanning the bottle barcode and then the specimen barcode.
viii. These all have an incubation time of 5 days for the bottles.

4. Aspirates from non-sterile sites e.g. wound drainage, pus and abscess material that are normally sent to the lab in a sterile container but arrive into the lab in a Blood Culture Bottle ONLY.

i. Treat the bottle as a specimen transport medium. Planting bench will inoculate the assigned media from aspiration of the bottle contents.

5. Aspirates from non-sterile sites e.g. wound drainage, pus and abscess material that are sent to the lab in a sterile container AND a Blood Culture Bottle.

i. Plant the specimen from the sterile container as usual and discard the blood culture bottle.

6. **Ascitis Fluid in Blood Culture Bottles ordered in UHN HIS as "ASCBT" test.**

i. Receive the order in LIS as in routine Bacteriology orders.
ii. Save the order and print the labels
iii. Apply the specimen labels on the bottles (the labels with the lab number that contains the extension 91).
iv. Load the bottles to Virtuo as usual by scanning the bottle barcode and then the specimen barcode.
v. (NO NEED to order "?BTLE – Fluid in BC Bottle")

Enterics

Faeces/Rectal Swabs

a) Direct Examination: Not routinely performed.
For special request, prepare a Gram stain for faecal leukocytes.

b) Culture:

Media	Incubation	
MacConkey Agar (MAC)	O_2,	35°C x 18 - 24 hours
Hektoen Agar (HEK)	O_2,	35°C x 18 - 24 hours
MacConkey Sorbitol Agar (SMAC)	O_2,	35°C x 18 - 24 hours
Campylobacter Agar (CAMPY)	Campy Jar	42°C x 48 hours
Selenite Broth (SEL) [1]	O_2,	35°C x 12-18 hours
If *Yersinia* is requested or patient is >1 month–12 years old (except for NICU), **add**:		
Cefsulodin Irgasan Novobiocin Agar (CIN)	O_2,	30°C x 48 hours
If *Vibrio* is requested, **add**:		
Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS)	O_2,	35°C x 18 - 24 hours
Alkaline Phosphate Broth (APB) [2]	O_2,	35°C x 5 - 8 hours
If *Pleiomonas* is requested, **add**:		
Blood Agar (BA)	O_2,	35°C x 24 hours
If *Aeromonas* is requested, **add**:		
Blood Agar (BA)	O_2,	35°C x 24 hours
Cefsulodin Irgasan Novobiocin Agar (CIN)	O_2,	35°C x 24 hours
If *Neisseria gonorrhoeae* (GC) is requested (rectal swab only), **inoculate only**:		
Martin-Lewis Agar (ML)	CO_2,	35°C x 72 hours

If *C. difficile* toxin assay is requested set up:		Cepheid GeneXpert PCR
For Mount Sinai Hospital patients: Specimen must also set up for Vancomycin Resistant Enterococcus (VRE) screen:		
Brilliance VRE Agar (BVRE)	O_2,	37°C x 36hrs in the dark

Notes: 1. Subculture Selenite broth following overnight incubation onto HEK. Incubate the sub-cultured HEK at 35°C in O_2 for 12 - 18 hours.

2. Subculture APB to TCBS Agar after 5-8 hours incubation. Planter has to notify the technologist on the Enteric bench at the time of processing. Incubate the TCBS Agar at 35°C in O_2 for 18 – 24 hours.

WASPLab Stool Processing:

Stool cultures received in Copan FecalSwab will be processed by the WASP and incubated within the

Processing Stool:

1. Upon receipt of specimens, put EPT specimens in a WASP palette marked for Stool Culture. The “test ordered” should only be for Stool C&S.
2. Palettes will be loaded onto the WASP at 9am, 2pm, 9 pm and 2 am. Fill the silos of the WASP2 with the appropriate plates prior to loading the palette.
3. Let WASP inoculate and streak the media and transfer the media plates to WASPLab O2 incubator. On the WASP, flip the orientation of the loop after stools are complete. Perform a wasp cycle on the loop.
4. The CAMPY plates will be sent to stacker 202 of the WASPLab line. Put the CAMPY plates into an offline incubation box, set up the box up with CAMPY gas pack. Incubate the box in the 42oC incubator.
5. Unload the Selenite broths, put them in a rack by their incubation time and incubate them in the 37oC.

Subculturing selenite broths:

1. Remove Selenite broths to subculture by the "load time" in the selenite rack (12 hrs after incubated). Eg. At 9am, load selenites that were set up at 9pm.
2. In the LIS:
    a. Open "Enterics" **Resulting Worklist**
    b. At the bottom left, click "Mark". Scan all selenite LIS barcodes
    c. On the right panel, click "Define MC (^M)". Type "SEL" and hit OK.
    d. From the keypad, select "^SFHEK". Select "OK"
    e. On the right panel, click "Add Result (^F7)". Exit worklist.
3. Load the selenites onto a palette and onto WASP2. Store selenites in a selenite done box. Keep 2 boxes worth. Discard older box when both are full.

Rectal/Large Bowel (Colon) Biopsies

a) Direct Examination: Gram stain not indicated.

b) Culture:

Media	Incubation
MacConkey Agar (MAC)	O_2 35°C x 18 – 24 hours
Hekton Agar (HEK)	O_2 35°C x 18 – 24 hours
MacConkey Sorbitol Agar (SMAC)	O_2 35°C x 18 – 24 hours
Camyplobacter Agar (CAMPY)	Campy Jar 42°C x 48 hours
Selenite Broth (SEL)	O_2 35°C x 18 – 24 hours

Genital Specimens

Cervical Swabs

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Chocolate Agar (CHOC)	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

If Group B streptococcus is requested, refer to the Group B streptococcus screen section.

Group B Streptococcus Screen

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Carrot Broth for Group B Strep (CAROT)	O_2, 35°C x 24 hours

Processing on the WASP:

1. Load wasp with CAROT broths
2. Load GBS specimens into the WASP
3. When WASP processing is complete, in the hood place one full tile into each CAROT broth.
4. Incubate CAROT broths.

Manual Processing: (when WASP is unavailable)

1. Inoculate 2-3 drops of each GBS specimen into each CAROT broth
2. Add one full tile into each CAROT broth.
3. Incubate CAROT broths.

c) Subculture:

Media	**Incubation**
Colistin Nalidixic Acid Agar (CNA)	O_2, 35^oC x 24 hours

<u>To Subculture Carrot Broths by Wasp:</u>

1. Gynae bench technologist will bring colourless Carrot broths to the planting area and process specimens on WASP themselves once WASP is unoccupied.
    a. First thing in the morning after cleaning is an ideal time
2. Ensure broth has WASP label. Otherwise, generate a large LIS barcode label and affix to swab.
3. Place broths in WASP **BLUE** palette(s) labelled 'BLU URISTRAW'.
4. Obtain adequate number of CNA plates for the broths being subcultured
5. Ensure that the WASP is not processing specimens before proceeding.

6. Take plates and palettes to the WASP:
    - Check status of WASP, by clicking on the "MAIN" icon on bottom toolbar
    - If "Paused", click "STOP", then "RESET".
    - Load palettes onto conveyor belt
    - If "READY", and there are plates to remove from the WASP, click "OPERATIONS", then click "UNLOAD CAROUSEL". Four stacks will appear on the screen. Click on each stack that has plates in it. Unload plates to a trolley. Click "OK". Otherwise, proceed to the next step
    - Click "CLOSE" (bottom right corner)
    - Click "SWITCH MODE"
    - Click "TECHNOLOGIST"
    - Click "SETTINGS" (icon on bottom toolbar)
    - Click "CAROUSEL" (icon in the middle of the screen)
    - Click "LOAD CONFIGURATION"
    - Click "GBS-CNA"
    - Click on the number on the carousel designated (6)

- Load CNA plates into the carousel (If you need to load more than the tower will hold, fill up to the max and the WASP will alert you when to add the remainder.)
- Click "OK"
- Click "Start" icon
- Click on the icon "COPAN 12"
- Using the mouse, slide the cursor down to you see the icon and highlight the icon of a wasp with "GBS-CNA")
- Click "OK"
- Load palettes
- WASP will begin processing

7. After subculturing is complete:

- Click "MAIN"
- Click "Stop"
- Click "Operations" ", then click "UNLOAD CAROUSEL". Four stacks will appear on the screen. Click on each stack that has plates in it. Unload plates to a trolley. Click "OK".
- Click "MAIN"
- Click "RESET"
- Click "SETTINGS" (icon on bottom toolbar)
- Click "CAROUSEL" (icon in the middle of the screen)
- Click "Load Configuration"
- Choose IC-UR-WND-BRUC-KV
- Click "OK"
- Click "Close"
- Click "SWITCH MODE"
- Click "LIS"
- Check bin for rejects. Sub those manually.
- Unload palettes

8. Return Carrot broths and SUBCN plates to the Gynae bench.

Vaginal Swab for Vaginitis/Vaginosis Screen

a) Direct Examination:

i. Wet mount: To be set up immediately. Gently press the swab into a drop of sterile saline on a slide. For Eswabs, vortex swab and add 2 drops on a slide.
Place a cover slip on the slide and examine under the microscope using the 40X objective. Examine for the presence of *Trichomonas vaginalis.*.

ii. Gram stain: Examine for the presence of yeast, clue cells and organisms associated with bacterial vaginosis.

Vaginal Swab for Culture

a) Direct Examination: not required

b) Culture:

Media	Incubation
Colistin Nalidixic Acid Agar (CNA)	CO_2, 35°C x 48 hours
Carrot Broth for Group B Strep (CAROT)	O_2, 35°C x 24 hours
Chocolate Agar (CHOC) (if GC is requested)	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML) (if GC is requested)	CO_2, 35°C x 72 hours
MacConkey (MAC) (for <12 years old)	CO_2, 35°C x 48 hours

Urethral Swab

a) Direct Examination: Gram stain - Quantitate the presence of pus cells and intracellular gram negative diplococci.

b) Culture:

Media	Incubation
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

Penis Swab

a) Direct Examination: Gram stain.

b) Culture:

Media	Incubation
BA	CO_2, 35°C, x 48 hours
MAC	O_2, 35°C, x 48 hours
CNA	O_2, 35°C, x 48 hours
CHOC*	CO_2, 35°C, x 72 hours
ML*	CO_2, 35°C, x 72 hours

*Occasionally, urethral swabs may be labelled as penile swabs. *Neiserria gonorrhoeae* culture is set up for this reason.

The ML should be inoculated by rotating the swab in a Z streak manner. The inoculum is then streaked by the ISOplater to obtain discrete colonies. Examine ML plates at 48 and 72 hours.

Seminal Fluid

Set up all specimens for culture and sensitivity testing as outlined below. After inoculating the culture media below, forward the remainder of the specimen to the Virology lab for molecular detection of GC and CT

a) Direct Examination: Not required

b) Culture:

Media	**Incubation**
Blood Agar (BA)*	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC) [ab]	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML) [ab]	CO_2, 35°C x 72 hours
MacConkey Agar (MAC)*	O_2, 35°C x 48 hours

*Use a 10 µl disposable culture loop to inoculate media by using urine colonies count culture technique.
[a]Dilute specimen 1:2 in a 15mL-conical tube with sterile saline before inoculating CHOC and ML agar . Store the original sample and the labelled conical tube with miscellaneous specimens.
[b]Use a sterile pipette apply one drop to media and streak

Endometrial Swabs, Biopsies and Curettings, Placenta Swab/Tissue,

Products of Conception, Endometrial/Uterine, Cul de sac/Transvaginal,

Fallopian Tube, Tubo-Ovarian Swabs or Aspirates

a) Preparation of specimen for culture:

1. If tissue is received, aseptically macerate the tissue with the use of a tissue grinder or stomacher.
2. If STAT TB is requested and approved by the Microbiologist, prepare a slide for Acid Fast Bacilli (FLUOROCHROME STAIN for MYCOBACTERIUM). A portion of the specimen should be forwarded to the Public Health Laboratory (PHL) for processing.
3. If TB culture is requested, send half of the specimen to PHL.

b) Direct examination: Gram stain.

c) Culture:

Media		***Incubation***
Blood Agar (BA)	CO_2,	35°C x 48 hours
Chocolate Agar (CHOC)	CO_2,	35°C x 48 hours
Martin-Lewis Agar (ML)	CO_2,	35°C x 72 hours
MacConkey Agar (MAC)	O_2,	35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)*	AnO_2,	35°C x 48 hours
Kanamycin-Vancomycin Agar (KV)*	AnO_2,	35°C x 48 hours
Fastidious Anaerobic Broth (THIO)*	O_2,	35°C x 48 hours

* If tissue/aspirate or anaerobic swab is received, add BRUC, KV and THIO.
For tissues and biopsies, freeze remaining tissue in the -70°C freezer for minimum of 3 months.

Genital Ulcer Swab

Refer to Ministry of Health Specimen Collection Guide

Intra-Uterine Device

a) Direct Examination: Gram stain of secretions.
Examine for the presence of branching gram positive bacilli suggestive of *Actinomyces* species.

b) Culture: Not indicated.

Infection Control Screening

Methicillin Resistant Staphylococcus aureus (MRSA) Screen

a) Direct Examination: Not indicated

b) Culture:
Routine Screening:

Media	Incubation
OXOID Denim Blue Agar (DBLUE)*	O_2, 37°C x 24 h -in the dark

If multiple swabs from a single patient are received individually, then process as separate specimens. If multiple swabs from a single patient are received as a "bundle" with a single label and order number, then process all swabs in the bundle on a single “DBLUE” plate.

On Fridays and Saturdays, specimens will not be planted past 3 pm. Any specimens received after this time will be held and planted the following morning.

These will be stored in a basket labelled for this purpose in the planting refrigerator.

Vancomycin Resistant Enterococcus (VRE) Screen

a) Direct Examination: Not indicated

b) Culture in non-outbreak setting:

Media	Incubation
Brilliance VRE Agar (BVRE)	O_2, 37°C x 48h in the dark

Inoculate the BVRE Agar by rotating the swab on the primary inoculum area to the size of 2.5 cm in diameter (size of a Loonie).

Put inoculated/streaked plates into the VRE bin in the incubator.

On Fridays and Saturdays, specimens will not be planted past 3 pm. Any specimens received after this time will be held and planted the following morning.

NB: In the event of an outbreak investigation, the above protocol (b) may not apply. VRE PCR maybe ordered on prior arrangement by Infection Control or Microbiologist. All swabs for VRE PCR are to be received by 8 am so they may be processed as a batch. VRE PCR positive specimens will be processed as per protocol (c) below.

c) Culture for **VRE PCR positive samples** in outbreak setting:

Media	**Incubation time (all O_2 at 37°C)**
i) Place 500uL (0.5 mL mark of transfer pipette) of the eSwab transport medium into:	
3 mL Brain Heart Infusion broth (BHIB)	overnight on shaker
Place 30uL (1 drop from transfer pipette) of the eSwab transport medium onto:	
Brilliance VRE Agar (BVRE)	48h in the dark
ii) If BVRE is no growth after 24 hours of incubation, subculture swab from BHIB to:	
Brilliance VRE Agar (BVRE)	48h in the dark

Resistant Gram Negative Bacilli Screen

a) Direct Examination: Not indicated

b) Culture:

Media	**Incubation**
For *Enterobacteriacae* with fluoroquinolone and/or aminoglycoside resistance but susceptibility to cefpodoxime:	
MacConkey Agar (Mac) –no antibiotic	O_2, 35°C x 18 h

For *Serratia marcescens* outbreaks, use MacConkey Agar + 7.5 mg/L colistin; in-house media preparation to be cleared by Microbiologist.

ESBL & CRE Screen

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
ESBL Isolation Agar - MacConkey agar with 2 µg/ml cefpodoxime (Media code: MCPOD)	O_2, 37°C x 18-24 hours

CRE Screen

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
ESBL Isolation Agar - MacConkey agar with 2 µg/ml cefpodoxime (Media code: MCPOD)	O_2, 37°C x 18-24 hours

RESISTANT Pseudomonas aeruginosa SCREEN

Specimen	Processing	Media	Incubation
Water	Centrifuge the entire sample at 3500 rpm for 20 minutes. Pour off all supernatant. Transfer the contents of a 2 mL tube of BHI	BHI Broth	O_2 at 35°C overnight

Specimen	Processing	Media	Incubation
	broth into in the falcon tube containing the sediment Subculture after overnight incubation to MCPOD by the IC Bench technologist	 MCPOD	 O_2 at 35°C for 24 hours
Environmental swabs	Incubate the BHI Broth Subculture BHI broth after overnight incubation to MCPOD by the IC Bench tech using a new sterile swab	 MCPOD	O_2 at 35°C overnight O_2 at 35°C for 24 hours
Patient pharmaceutical infusates/injectables	≤1 mL >1 mL	TH14 SD14 ETH14 ESD14	O_2 at 35°C for 14 days O_2 at RT° for 4 days O_2 at 35°C for 14 days O_2 at RT° for 4 days
Swabs from patients	Directly inoculate MCPOD plate with specimen	MCPOD	O_2 at 35°C for 48 hours

Mycology

1. All specimens, except for skin scrapings, nail and hair (see below) are processed initially in the general planting section of the Microbiology Laboratory. Requests for skin scrapings, nails and hair should be forwarded to the Public Health Lab (PHL) for processing. Fungal smears and inoculated culture plates should be forwarded to the Mycology section for staining, interpretation, incubation and work-up.

2. **Fungal smears:**
   i) At least one, and where applicable, two smears from each specimen should be prepared and forwarded to the mycology section. One smear is for fungal staining and the second is held in reserve for special stains (if indicated).
   ii) In the planting area, the smears should be fixed in 99.9% methanol for 5 minutes before staining.
   iii) Fungal smears should be stained using Fungi-Fluor™ (Refer to Staining Methods).

3. **Culture:**
   i) Refer to Bacteriology manual and/or Table 1 below, for appropriate media to be inoculated depending on specimen type. Between 0.1and 0.5 mL of fluid specimens is recommended for each media.
   ii) Tissue specimens should be mixed with filtered sterile saline. Gently cut the tissue with a sterile scalpel and use sterile forceps to inoculate the media
   NB: DO NOT grind the specimen or put it into the Stomacher to maintain viability of possible fungi including Zygomycete .
   iii) Sterile body fluids and urine specimens should be centrifuged at 3500 rpm x 20 min prior to inoculating them. The supernate should be removed leaving sufficient volume to resuspend the sediment and inoculate the appropriate number of culture media.
   iv) Inoculated culture plates should be held unsealed overnight at room temperature to dry the plates. The following morning, all plates should be placed in a plastic bag and sent to Mycology to be sealed with Parafilm to prevent contamination and incubated at 28^{o}C.

Table 1 - PROCESSING AND INCUBATION OF SPECIMENS FOR MYCOLOGY

SPECIMEN	FS	IMA	EBM	BHIM	SAB G	MYCOSEL	Incubation[4] O_2, 28°C
Bone Marrow[5]	X	X	X	X			4 weeks
Brain Biopsy/Abscess	X	X	X	X			4 weeks
Sterile Fluids:							
CSF[1]		X	X	X			4 weeks
Pleural Fluid	X	X	X	X			4 weeks
Synovial Fluid	X	X		X			4 weeks
Pericardial/Ascitic Fluid	X	X		X			4 weeks
Peritoneal/Dialysis Effluent[2]	X	X		X			4 weeks
Aqueous/Vitreous	X	X		X			4 weeks
Eye/Corneal Ulcer	X	X					3 weeks
Blood Culture[3, 5]		X	X	X			4 weeks
Lung Biopsy	X	X	X	X			4 weeks
Tissue Biopsy	X	X		X			4 weeks
Lymph Node	X	X	X	X			4 weeks
Skin Biopsy	X	X		X			4 weeks
Clot/Thrombus	X	X		X			4 weeks
Bone/Joint	X	X		X			4 weeks
Heart Valves	X	X	X	X			4 weeks
Intravascular Grafts	X	X	X	X			4 weeks
Abscess/Pus/Wound[2]	X	X		X			4 weeks
Autopsy	X	X					3 weeks
Respiratory Secretions:							
Sputum[2]	X	X	X	X			4 weeks
Tracheal Aspirate[2]	X	X	X	X			4 weeks
Bronchial Washing	X	X	X	X			4 weeks
BAL (Routine)	X	X	X	X			4 weeks
BAL(Lung Transplant Screen)	X	X	X	X			4 weeks
Sinus/Antral Wash	X	X					3 weeks
Nose[2]		X					3 weeks
Mastoid	X	X					3 weeks
Ear	X	X					3 weeks
Urine[2]		X	X				3 weeks

1. Cryptococcal Antigen in the Planting Area
2. Only if Fungal Culture is specifically requested
3. Collected in "Isolator 10" Tube
4. If dimorphic fungus is requested, regardless of site, media should be incubated for 6 weeks.

5. For Malassezia request, incubate IMA And IMAO for one week Only

FS	Fungal Stain
IMA	Inhibitory Mould Agar
IMAO	Inhibitory Mould Agar with Chloramphenicol, sterile olive oil to be overlaid at Mycology bench.
BHIM	Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide
EBM	Aesculin agar with chloramphenicol, gentamycin
SAB G	Sabouraud Dextrose Agar
MYCOSEL	contains Chloramphenicol and Cyclohexamide
PDA	Potato Dextrose Agar

Respiratory Specimens

Bronchoalveolar Lavage (BAL)

a) Specimen Processing

Bronchoalveolar Lavage specimens from the same patient should be pooled together and processed as a single sample EXCEPT specimen from Trillium Gift of Life Donor (identified by MRN prefixed with "DONOR")

BAL Test specimen requirement if requested*:

- 1mL for bacteriology culture
- 1 mL for fungus (if requested OR specimen is from lung transplant patients)
- 5 mL for TB (if requests, send to PHL)
- 2 mL for Virology (if requested) - Supernatent
- 1 mL for Legionella (if requested, send to PHL)
- 1 mL for *Chlamydia pneumoniae* (if requested, send to PHL)
- 1 mL for Galactomannan - Supernatent

C&S

1. Prepare a bacterial C&S gram smear with unspun specimen using a Cytospin funnel.

2. For bacterial culture requests, process specimens with liquefying solution before placing on the WASP for processing using 1mL loop.

   During WASP downtime: process liquefied specimen and inoculate media manually using a calibrated 1 uL loop to inoculate unspun sample onto each routine bacteriology plates below. Streak the loop down the center of each of the first set of plates and then cross-streaked at a 90^{o} angle to the inoculum.

Other requested tests processing

If the remaining specimen volume is less than the amount the requested tests volumes, call the physician to determine the priority of test(s) to be performed. Result the test(s) that cannot be done as "Insufficient sample for _____." Document in the LIS call window the name of the physician contacted.

1. Centrifuge the entire specimen at 3500 rpm for 20 minutes.

2. Remove all but 0.5 mL of the supernatant to a new sterile container labelled as "Supernatant".

3. Vortex the sediment to mix well.

4. Prepare smears (for Fungifluor stain and PCP IFA if tests are requested, respectively). See Direct Examination Preparation below.

5. Split/aliquot specimen for remaining tests requested requiring supernatant. Reconstitute the rest of the sediment with 1-10 mL of reserved supernatant for remaining requested tests. See test requirements above.

b) Direct Examination Preparation:

Stain the prepared smears as follows:

- Cytospin smear for Gram stain. Following Gram Stain procedure and deliver stained slide to the Gram Stain Bench.

- Concentrate smears (for Fungifluor stain and PCP IFA if C&S, fungus culture and PCP are requested respectively):
  - Forward slide to Mycology Section for Fungifluor staining and interpretation (if fungal culture is requested).
  - Forward slide to Virology Section for PCP IFA staining and interpretation (if PCP is requested).

c) Culture Preparation:

Inoculate the specimen onto the following media:

Media	**Incubation**
Inoculate with **unspun** specimen using **1 uL loop**:	
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add**:	
OF Base, Colistin, Bacitracin & Lactose Agar (OCBL)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates	CO_2, 35°C x 5 days

Inoculate with the **resuspended** specimen:		
If Fungus is requested OR specimen is from lung transplant patients, **add**:		
Inihibitory Mold Agar (IMA) *	O_2,	28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2,	28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2,	28°C x 4 weeks
If Nocardia is requested, **add**:		
Sodium Pyruvate Agar (PYRA)	O_2,	35°C x 4 weeks

* Forward inoculated fungal media to Mycology Section for incubation and work-up.

CMV Surveillance Bronchoscopy Specimens

Specimens for **routine CMV surveillance** require CMV culture only. Refer specimens to Virology immediately upon receipt.

Bronchial Brush Specimens

All brushes received in small cap (bijou) bottles in 1 mL Ringer's lactate solution should be processed "STAT" because colony counts may change if processing is delayed.

If the brush is received in <1 mL of fluid, add sufficient Ringer's lactate solution to make up 1 mL. Document in the “Test Comment” field of the LIS as “Brush received in wrong volume of fluid”.

If a dry brush is received, add 1 mL of Ringer's lactate solution into the vial. Document in the “Test Comment” field of the LIS as “Dry brush received”.

Mix the vial well by vortexing vigorously.

Measure 0.01 mL of the sample (using a calibrated **10 uL loop**) and inoculate onto routine media for culture of aerobic bacteria. Streak the loop down the center of the plate and then cross-streaked at a 90° angle to the inoculum top to bottom.

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation	
Blood Agar (BA)	CO_2,	35°C x 48 hours
Haemophilias Isolation Medium (HI)	CO_2,	35°C x 48 hours
MacConkey Agar (MAC)	CO_2,	35°C x 48 hours

If *B. cepacia* is required or specimen is from a patient with Cystic Fibrosis, **add**:

Of Base, Colistin, Bacitracin & Lactose Agar (OCBL)	O_2,	35°C x 5 day
Keep the BA, HI and MAC plates	CO_2,	35°C x 5 days

Epiglottal Swabs

a) Direct examination: Not indicated

b) Culture:

Medium	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours

Open Lung/Transthoracic Needle/Transbronchial Lung Biopsies/Lung Aspirates

a) Direct examination: Using clean, sterile slides make 3 touch preparation smears from a cut surface of the biopsy for:

i) Gram stain: Examine for presence of organisms and cells.

ii) Fungifluor stain: Forward to Mycology Section for staining and interpretation.

iii) Extra smear held in Mycology Section for special stains.

Macerate (using a hand grinder) the remaining biopsy tissue using sterile, saline as the diluent.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Fastidious Anaerobe Agar (BRUC)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days
Inhibitory Mold Agar (IMA) *	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM) *	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 28°C x 4 weeks
If *B. cepacia* is requested or the specimen is from a patient with Cystic Fibrosis, **add**:	
OF Base, Colistin, Bacitracin & Lactose Agar (OCBL)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates for 5 days	
If Nocadia is requested, **add**:	
Sodium Pyruvate Agar (PYRA)	O_2, 35°C x 4 weeks

***** Forward inoculated fungal cultures to Mycology for incubation and work-up.

Mouth Swabs

Applies to both C&S and Fungus test requests:
a) Direct Examination: Gram stain
b) Culture: Not indicated.

Nasal Swabs

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
If C&S is requested: Colistin-Nalidixic Agar (CNA)	CO_2, 35°C x 48 hours
If *N. meningitidis* is requested, **add**: Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
If MRSA is requested: OXOID Denim Blue Agar (DBLUE)*	O_2, 37°C x 24 h -in the dark

Nasopharyngeal Swabs/Auger Suctions for Bordetella pertussis

The specimen should be forwarded to the Public Health Laboratory (PHL) for processing.

Oral Abscess Swabs

If an anaerobic swab is received and *Actinomyces* is not requested, process for aerobic culture only. Do not process for anaerobic culture. Document in the "TEST Comment" field in the LIS as "Inappropriate specimen for anaerobic culture, therefore not processed"

a) Direct Examination: Gram stain

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours

If *Actinomyces* requested or suggested on Gram stain, **add**:

Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 7 days
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 7 days

Sinus/Antral Specimens

a) Direct examination:
   i) Gram stain - Examine for and quantitate the presence of pus cells and organisms.
   ii) Fungifluor - Forward to Mycology Section for staining and interpretation.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
Inhibitory Mold agar (IMA)*	O_2, 28°C x 3 weeks

If anaerobic culture requested, **add**:

Kanamycin Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 48 hours

*Forward inoculated fungal media to Mycology section for incubation and work-up.

Sputum including Endotracheal tube and Tracheotomy Specimens, Bronchoscopy Aspirates/Washings

a) Specimen preparation

- For sputum bacterial culture requests, process specimens with liquefying solution before placing on the WASP for processing. During WASP downtime, use liquefied specimen to inoculate manually.

- Flush small volumes of endotracheal tube (ETT) secretions from suction tubings of neonates with a small volume of WASP solution and transfer to a WASP tube for processing on the WASP

- An ETT tip from an adult is an unacceptable specimen and will not be processed. Notify the ward immediately and issue a report stating "ETT tips are not acceptable for culture".

- Bronchoscopy Aspirates/Washings from the same patient should be pooled together and processed as a single sample EXCEPT specimen from Trillium Gift of Life Donor (identified by MRN prefixed with "DONOR"). These samples are processed in the same manner as sputum

- For requests for viruses, forward sample to the Virology section for processing.

- Requests for "**STAT**" acid-fast bacilli (AFB) staining must be approved by a medical microbiologist or infection control practitioner before processing in-house. A portion of the sample should also always be forwarded to the Public Health Laboratory for processing.

- If mycobacterium (TB) is requested, forward a portion of the specimen to the Public Health Laboratory (PHL) for processing.

- If Legionella is requested, forward a portion of the specimen to the Public Health Laboratory (PHL) for processing.

- For Bronchoscopy Aspirates/Washings from **Donors or ICU**, process as BAL. Add Test Comment in both Gram and Culture results:
  This specimen }BRON
  "This specimen was received labelled as bronchial washing but was worked up as BAL"

b) Direct Examination:

Gram smears:
Prepared by the WASP except during downtime.
During WASP downtime select the most purulent portion of the specimen for gram staining.
Follow Gram Stain procedure and deliver stained slide to the Gram stain bench.

Approved requests for **STAT** acid fast/TB requests:
- See *Mycobacterium tuberculosis* & Rifampicin resistance PCR by Cepheid GeneXpert.
- For smears forward to PHOL.

Fungus requests:
Prepare smear for Fungifluor and forward to Mycology Section for staining and interpretation.

c) Culture:

Prepared by the WASP except during downtime.
During WASP downtime, select the most purulent portion of the specimen for plate inoculation.

Specimens will be inoculated onto the following media:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add**:	
OF base, colistin, bacitracin & lactose Agar (OCBL)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates	CO_2, 35°C x 5 days
If Nocardia culture is requested, **add**:	
Sodium Pyruvate Agar (PYRA)	O_2, 35°C x 4 weeks
If fungal culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 28°C x 4 weeks

* Forward inoculated fungal media to Mycology section for incubation and work-up.

Throat Swabs

a) Direct Examination: Not indicated for Group A streptococcus, *Neisseria gonorrhoeae* or *Neisseria meningitidis*.

If yeast (thrush) is suspected / requested: Gram stain. Examine for presence of pseudohyphae and/or budding yeast.

If Vincent's angina is suspected / requested: Gram stain. Examine for presence of spirochetes and/or fusiform bacilli and pus cells.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	AnO_2, 35°C x 18-24 hours
If *Neisseria gonorrhoeae* requested, **add**: Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
If *Corynebacterium diphtheriae* requested, forward swab to Public Health Laboratory (PHL) for processing.	

Note: The ML plate is inoculated by rolling the swab in a "Z" pattern over the medium followed by cross streaking with a sterile loop over the entire plate.

Set up anaerobic jar at 1600 hours and at the end of the evening shift.

Gastric Aspirates/Biopsies (for Helicobacter pylori)

a) Direct Examination:

i) Use one half of the tissue to prepare a smear for Gram stain and to directly inoculate a urea slant.

ii) Incubate the urea slant at 35°C for 1 – 4 hours.

iii) Macerate the remaining tissue for culture:

b) Culture:

Media	**Incubation**
Blood Agar (BA)	Microaerophilic, 35°C x 7 days
Campylobacter Agar (CAMPY)	Microaerophilic, 35°C x 7 days
Urea (Rapid)	O_2, 35°C x 4 hours

Gastric Aspirates/Swabs from Neonates or Stillborn

a) Direct Examination: Gram Stain

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

Respiratory Specimen Treatment Solutions:

Liquefying solutions are ready-to-use mucolytic agents for liquefying respiratory specimens prior to planting and streaking for the isolation of bacteria and fungi that cause respiratory tract infections.

Copan Sputum Liquefying (SL) Solution Procedure

1. Take one tube containing 1 ml SL solution and label with a LIS collection label.
2. Using the Copan Transfer Device, transfer an equal amount of specimen (~1mL) to SL solution into the tube.
   Note: best results can be achieved by transferring the sample into the solution in a 1:1
3. Dip and rotate the device in the medium 5-10 times.
4. Break off the tip end of the dipper and re-cap tube.
5. Vortex in the test tubes for 30 seconds at 2000/2500 rmp.
6. Leave the test tube at room temperature for at least 15 minutes. A prolonged contact time up to 6 hours doesn't impair the survival of bacteria or fungi contained in the sample and may be necessary for especially viscous samples.
7. Place tube onto the WASP. During WASP downtime for mucoid or aseptically remove aliquots of the liquefied sample and inoculate onto appropriate bacteriology culture medium.

Sterile Fluids

Cerebrospinal Fluids

NB: If only VDRL is requested, the specimen will **NOT** be cultured. All other requests require C&S. These specimens must be processed immediately.

Unspun sample is required for Biofire meningoencephalitis panel

1. Examine the fluid for colour, xanthochromia, turbidity and the presence of visible blood or clots. Record the observations in the LIS under COMMENT in the SOURCE screen.

2. If any of the following is requested:
   Cryptococcus or cryptococcal antigen,
   India ink preparation or
   Fungal culture
   Perform a cryptococcal antigen test on the supernatant or from the original specimen (see Technical Manual)

3. If bacterial antigens are requested, this test is no longer available.

If >1 mL of specimen is received:

4. Make a direct cytospin smear using 2-3 drops of unspun CSF. Set cytospin to centrifuge for 900 rpm for 3 minutes.

5. Centrifuge the remaining specimen at 3500 rpm x 20 minutes.

6. Transfer the supernatant to a sterile tube which can be used for biochemical, viral, molecular, Cryptococcal antigen or serological tests if required. Label clearly remaining supernatant and refrigerate.

7. Use the sediment to inoculate the culture media listed below. Label clearly any remaining sediment and refrigerate.

8. If Mycobacteria (TB) is requested, reconstitute sediment with 1 mL of supernatant and label as such. Forward the specimen to the Public Health Laboratory (PHL) for processing.

If <1 mL of specimen is received:

4. Make a direct cytospin smear using 2-3 drops of unspun CSF. Set cytospin to centrifuge for 900 rpm for 3 minutes.

5. **DO NOT** centrifuge the specimen. Consider calling other departments for more specimen. Consult a Microbiologist if the sterility of the extra specimen is questionable.

6. If more than one test is ordered, call the physician to prioritize the test ordered.

7. For routine C&S, prepare a Gram smear using a drop of CSF and then directly inoculate the culture media listed below.

8. If Mycobacteria (TB) is requested, forward a portion of the specimen to the Public Health Laboratory (PHL) for processing.

9. If virology is requested, forward a portion of the specimen to the Virology Section for processing.

10. If a swab is received,
    a. in the Order/Entry Specimen QA Comment field of the LIS as "UNST"
    b. in the Result Comment field enter "Swab received – insufficient specimen. A negative result may not rule out infection."

    Process the swab as per routine.

A. Direct Examination:

Gram stain

Make 2 smears if the specimen is grossly bloody.

B. Culture:

Media	**Incubation**
Blood Agar	CO_2, 35°C x 48 hours
Chocolate Agar	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth	O_2, 35°C x 5 days
If fungus or cryptococcus is requested, **add**:	
Inhibitory Mould Agar[1]	O_2, 28°C x 4 weeks
Esculin Base Medium[1]	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)[1]	O_2, 28°C x 4 weeks

[1]Forward inoculated fungal media to Mycology section for incubation and work-up.

Other Sterile Fluids – Amniotic, Pleural (Thoracentesis/Empyema), Peritoneal (Ascites), Synovial (Joint), Pericardial, Tympanocentesis, Intraocular, Hydrocele Fluids etc

NB: Chest tube drainage will be treated as a wound specimen (Refer to Wound/Tissues/Aspirates Culture Manual).

1. If a clotted specimen is received, emulsify the coagulated material using a sterile swab or loop.

2. If >1 mL of fluid is received, centrifuge the specimen (3500 rpm x 20 minutes). If <1 mL of specimen is received, do **NOT** centrifuge the specimen.

3. Transfer the supernatant into a sterile container and refrigerate until the final report is issued. Label the container as supernatant.

4. Use the sediment to prepare the smears and inoculate the culture media. If <1 mL of specimen is received, prepare smears and inoculate culture media directly.

5. If viral culture is requested, forward a portion of the specimen to the Virology Section for processing.

6. If Mycobacteria (TB) is requested, forward a portion of the specimen to the Public Health Laboratory (PHL) for processing.

7. If a swab is received,
   a. In the Order/Entry Specimen QA Comment field of the LIS as "UNST"
   b. In the Result Comment field enter "Swab received – insufficient specimen. A negative result may not rule out infection."

   Process the swab as per routine.

A. Direct Examination: Gram stain
Calcofluor white stain (If fungus is requested).

B. Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 2 days
Chocolate Agar (CHOC)	CO_2, 35°C x 2 days

Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[2]	AnO_2, 35°C x 48 hours
For sterile fluids other than Peritoneal (Ascites) fluid:	
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days
For Peritoneal (Ascites) fluid:	
Blood Culture bottles (FA and FN)	BacT/Alert 35°C x 5 days
If fungus is requested, **add**:	
Inhibitory Mould Agar (IMA) [1]	O_2, 28°C x 3 weeks
Esculin Base Medium (EBM) [1]	O_2, 28°C x 3 weeks

[1] Forward inoculated fungal media to Mycology section for incubation and work-up.
[2] Not needed for **any** Eye fluids or Tympanocentesis specimens

Peritoneal Dialysis Effluent

NB: No more than one dialysis fluid per patient should be processed every other day. If a bag of cloudy fluid is received after a clean one is processed, culture and sensitivity is always done. A portion of the fluid should be sent to Hematology for cell count if requested.

1) Examine the bags noting the colour and turbidity of the fluid. Record the observations in the LIS under COMMENT in the SOURCE screen.

2) Shake bag well to mix. Working at the sink, disinfect the port with 70% ethanol and let stand for several minutes. Open clamp and run off some of the dialysate.

3) Fill a red top vacutainer tube with approximately 5 mL of dialysate and send to Hematology for cell count.

4) Fill three 50 mL sterile plastic centrifuge tubes with dialysate. Centrifuge two of the tubes (3500 rpm x 20 minutes). Store the third centrifuge tube at 4°C for at least 7 days. Discard the bag after collecting dialysate.

5) Decant the supernatant leaving approximately 10 mL in each tube. Vortex well to resuspend the sediment and pool the two tubes into one. Prepare Gram smear and inoculate culture media as outlined below.

a) **Direct Examination**:
   i) If specimen is cloudy: Gram stain
   ii) If specimen is clear: Gram stain is not needed.

b) **Culture**:

Media	Incubation
If specimen is clear:	
Bact/Alert bottles*	Processed as per blood culture protocol
If specimen is cloudy:	
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
BacT/Alert bottles*	Processed as per blood culture protocol

*Inoculate both an aerobic and anaerobic bottle with 8 to 9 mL of fluid each.

Predialysis Fluid

1. Examine the fluid noting the colour and turbidity. Record the observations in the LIS under COMMENT in the SOURCE screen.

2. Centrifuge specimens (3500 rpm x 20 minutes).

3. Decant supernatant leaving approximately 2-3 mL. Vortex well to resuspend the sediment.

    a) Direct Examination:

        i) If specimen is cloudy: Gram stain
        ii) If specimen is clear: No Gram stain is needed.

    b) Culture:

Media	Incubation
If specimen is clear:	
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 4 days
If specimen is cloudy:	
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

Bone Marrow (Aspirates or biopsies)

1. If SPS tube received, inoculate to media listed below

For bone marrow specimens not received in SPS

- If <1 ml is received, sterile saline may be added to a final volume of 1 ml so that the media listed below can be inoculated.
- If a clotted specimen is received, emulsify the coagulated material using a sterile swab or loop.
- If only heparinized sample is received and bacterial or fungal C&S is requested, we can process it with the comment:

"This specimen was received in a heparin tube which may suppress bacterial growth leading to a false negative result. Please take this into consideration when interpreting results"

- A portion of **ALL** specimens should be forwarded to the Public Health Laboratory (PHL) for Mycobacteria (TB) culture. (There is no limitation of using heparin for TB per the PHOL website and Canadian Tuberculosis Standards)

a) Direct Examination: Gram stain.
Calcofluor white stain.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidous Anaerobic Broth (THIO)	O_2, 35°C x 5 days
Inhibitory Mould Agar (IMA)[1]	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM)[1]	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)[1]	O_2, 28°C x 4 weeks

[1]Forward inoculated fungal media to the Mycology Section for incubation and work-up.

Blood, Platelets, & Other Transfusion Products

A sample of the transfusion product from the bag should be processed. Order entry into the LIS

- Open the seal of an unused port and insert a sterile spike
- Remove cap on spike outside the bag and attached a 10mL syringe
- Aspirate sample for processing to direct agar/gram smear and a maximum of 10mL in each blood culture bottle as below:
    - For volumes greater than 20mL
        - Aspirate 10mL of sample, attached needle to syringe, inject 10mL of sample into blood culture bottle. Discard syringe
        - With a new sterile syringe, aspirate another 10mL of sample, attached needle to syringe, inject 10mL of sample into blood culture bottle
        - With a new sterile syringe, aspirate 1mL of sample for inoculation of gram and direct agar
    - For volumes 1ml-20mL
        - Follow the procedure for >20mL using 1mL to inoculate the gram/direct agar and 1mL split into both blood culture bottles
    - For volumes <1mL
        - Inoculate gram / direct agar. If any remaining volume, inoculate a THIO broth.

a) Direct Examination: Gram Stain.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
FAN Aerobic Blood Culture bottle (FO2)*	in BacT/Alert 35°C x 5 days
FAN Anaerobic Blood Culture bottle (FN)*	in BacT/Alert 35°C x 5 days

*Inoculate 10 ml of product from blood bag into each blood culture bottles

*If <10 ml of product received, aseptically inject 10 to 20 cc of Tryptone Soya broth into the blood bag, the bag shaken and the broth re-aspirated and aseptically inoculated into the blood culture bottles

Handling of Body Fluids in Blood Culture Bottles

Urines

a) Direct Examination:

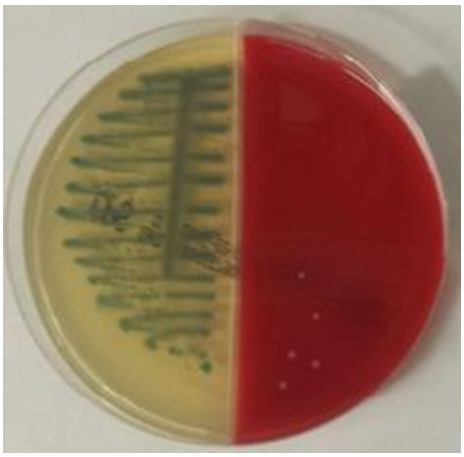
Gram stain: Not routinely performed. If specifically requested, perform Gram stain directly on unspun specimen.

Fungal stain: Not routinely performed. If dimorphic fungus or cryptococcus specifically requested, prepare 2 slides with spun urine (3500 rpm x 15 min.). Send unstained slides to mycology for staining and interpretation.

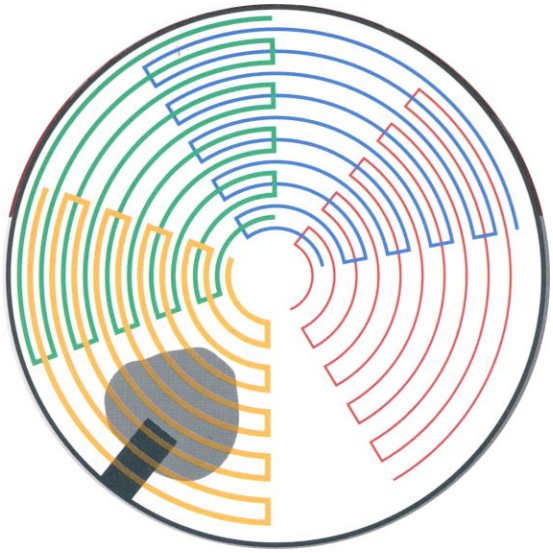
Eosinophil stain: Not routinely performed. If requested, prepare smear by cytospin method and fix in absolute alcohol. Stain slide as per Technical manual.

b) Culture:

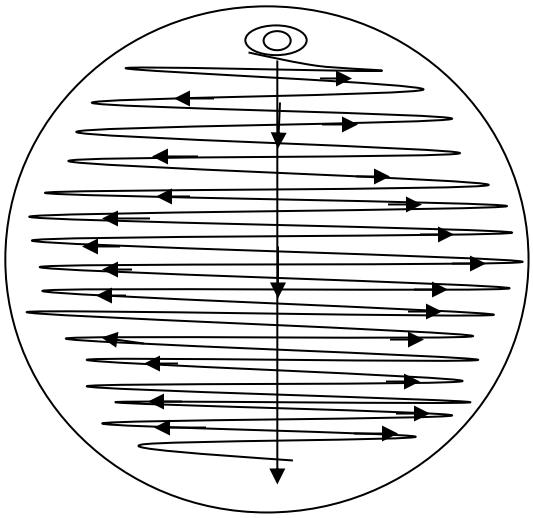
Automatic WASP culture method: Place Urine ChromID CPS4/CNA bi-plate in WASP culture machine and perform urine culture in WASP.
WASP mix the urine specimen and dip a sterile calibrated 0.001 mL or 0.01 mL loop vertically into the sample. Streak the loop down the center of the plate and then cross-streaked at a 90^{o} angle to the inoculum. (See diagram below).



Manual method: Mix the urine specimen and dip a sterile calibrated 0.001 mL or 0.01 mL loop vertically into the sample. Streak the loop down the centre of the plate and then cross-streaked at a 90^{o} angle to the inoculum. (See diagram below). If using the Autostreaker, inoculate the plate with a small streak using a sterile calibrated loop (See diagram below). The amount of urine inoculated is dependent on the type of urine specimen that is processed. (See Table below)



i) Autostreaker



ii) Manual inoculation

Urine Hold Specimens:

Mount Sinai Hospital urine samples from 11S, 11N, 14N, 14S and 14 Step Down will have urines ordered from Cerner as group test "UCHX1 - Urine Hold".

- Urine Hold specimens seen or loaded in to the WASP and rejected.
- Go to Order Entry and Receive the specimen.
- Leave Plated blank
- In 2 hours the LIS will automatically add the canned message }UCH1 and give the Test a Preliminary status.

  "The majority of positive urine cultures from inpatients without an indwelling urinary catheter represent asymptomatic bacteriuria. If you strongly suspect that your patient has developed a urinary tract infection, please call the Microbiology laboratory within the next 48 hours to have this specimen processed. If a phone call is not received, this specimen will not be processed."

A. If a request is made to process a urine sample:

- Document call
- In SoftMIC Result Entry, Remove Urine Hold comment "The majority of positive urine…"
- Result test code UCH1with canned message from Keypad **}req - Request for culture received, results to follow.**
- Finalize Urine Hold test UCHI.
- In order entry use the same LIS order as the Urine Hold order.
- Select "MSU" from order entry keypad and Save.
- Load specimen onto the WASP for routine urine processing.
- The WASP will add the Plated time.

B. If NO request is made, urine hold test will automatically be Finalized.

TABLE 1. Specimen type, inoculum, media and incubation conditions.

Media	Incubation
Voided urine: use 0.001 mL loop ChromID BUTI/CNA bi-plate	O_2 35°C x 18 – 24 hours
In and Out catheter/catheter insertion urine: use 0.001 mL loop ChromID BUTI/CNA bi-plate	O_2 35°C x 18 – 24 hours
Aseptically collected urine: use both 0.01 mL and 0.001 mL loop ChromID BUTI/CNA bi-plate Label the plates with the inoculum volume	O_2 35°C x 18 – 24 hours
Suprapubic Bladder Aspirate: use both 0.01 mL and 0.001 mL loop ChromID BUTI/CNA bi-plate Label the plates with the inoculum volume	O_2 35°C x 48 hours O_2 35°C x 48 hours AnO_2 35°C x 48 hours
Segmented urines labeled as VB1, VB2, VB3: use both 0.01 mL and 0.001 mL loops (2 sets) ChromID BUTI/CNA bi-plate Label the plates with the inoculum volume.	O_2 35°C x 18 – 24 hours
Prostatic Fluid labeled as EPS sent together with VB1, 2 or 3 or labeled as Seminal Fluid sent together with VB1, 2 or 3: use both 0.01 mL and 0.001 mL loops (2 sets) ChromID BUTI/CNA bi-plate Label the plates with the inoculum volume.	O_2 35°C x 18 – 24 hours
Urine for routine fungus: use 0.001 mL loop ChromID BUTI/CNA bi-plate Label the plates with the inoculum volume.	Room temperature x 18-24 hours

Media		Incubation
Urine for Cryptococcus or specific filamentous fungi: centrifuge urine at 3500 rpm, for 15 minuntes		
	O_2	28°C x 3 weeks.
EBM	O_2	28°C x 3 weeks.
IMA		
Inoculate 1 drop of sediment per plate. Forward plates to Mycology		

Note: For specimens processed after 1600 hours, place the plates in a separate basket in the incubator.

Special Requests:

Eosinophil Stain –

Not routinely performed. If requested, prepare smear by cytospin method and fix in absolute alcohol. Stain slide as per Technical manual.

Bacterial Latex Agglutination –

Not done due to poor sensitivity and specificity.

Anaerobes –

Appropriate specimen is bladder suprapubic aspirate – see above Table. Process these specimens immediately. If requested on other urines, consult the Medical Microbiologist.

Chlamydia Detection –

Send the specimen to Virology immediately.

Legionella Antigen Detection –

Send the specimen to the Provincial Health Laboratory.

Leptospira Detection –

Send the specimen to the Provincial Health Laboratory.

Cryptococcus/Systemic Fungi –

See above Table.

TB Culture –

Send the specimen to the Provincial Health Laboratory.

Viral Culture –

Send the specimen to Virology immediately.

Parasitology – *Schistosomiasis* –

Send the specimen to Parasitology immediately.

Wounds/Tissues/Aspirates

Duodenal or Small Bowel Aspirate/Swab/Biopsy

a) Direct Examination: Gram stain not performed.

b) Culture:

i) Duodenal or Small Bowel Aspirates
Process Duodenal and Small Bowel aspirates for O&P only. Forward the specimen to the Parasitology section immediately. If a delay is anticipated and the specimen is not received in SAF, transfer the specimen to SAF. DO NOT process these specimens routinely for bacterial culture unless requested.

ii) Duodenal or Small Bowel Swab

Media	Incubation
Blood Agar (BA)	O_2, 35°C x 18 - 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 18 - 24 hours
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours

Intraoperative/Interventional Swabs

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Calcofluor white stain - Refer to Mycology Manual.

b) Culture:

Media	**Incubation**
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)[1]	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[1]	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)[1]	O_2, 35°C x 5 days
If anaerobic swab is received, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
If fungus is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Esculin Base Medium (EBM)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

* Forward fungus culture media to Mycology section for incubation and processing.

Wound/Abscess Swabs and Drain

a) Direct Examination: Gram stain - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours
For chest tube drainage and tracheal swabs, **add**:	
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
If anaerobic transport media is received, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours

Bite Wound Swabs

a) Direct Examination: Gram stain – Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
If anaerobic transport media received, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours

Intravenous & Central Line Catheter Exit Site Swabs

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

Intraoperative/Interventional Abscess (Pus, Cyst Fluid or Aspirate)

1. If >1 ml of thin fluid is received, centrifuge specimen at 3500 rpm for 20 minutes. For purulent and thick specimens or if <1 ml is received, centrifugation is not required.

2. If parasitology is requested, send a portion of the fresh specimen to the Public Health Laboratories (PHL) on Mondays to Fridays. On weekends and holidays, mix an equal volume of specimen with SAF and send to the Public Health Laboratories (PHL). Note on the specimen label that it has been mixed with SAF.

3. If TB culture is requested, send a portion of the specimen to the Public Health Laboratory (PHL) for processing.

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Calcofluor white stain - Refer to Mycology Manual.

b) Culture:

Media	**Incubation**
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)[1]	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[1]	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)[1]	O_2, 35°C x 5 days
If fungus is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Esculin Base Medium (EBM)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

* Forward fungus culture media to Mycology section for incubation and processing.

Pus & Abscess Material (other than Intraoperative/Interventional, Rectal or Bartholin)

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.
Modified acid fast stain - If Actinomyces or Nocardia is requested or suggested on Gram stain.
Calcofluor white stain - If fungus is requested. (Refer to Mycology Manual).

b) Culture:

Media	**Incubation**
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[1]	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)[2]	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[2]	AnO_2, 35°C x 48 hours
If Nocardia is requested, **add**:	
Na Pyruvate Agar (PYRU)*	O_2, 35°C x 4 weeks
AND fungus media below	
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

*Forward the fungus culture media and PYRU to the Mycology section for incubation and work-up.

NOTE:
1. If Nocardia is requested, send the BA and CHOC plates to mycology after 48 hours incubation. The plates will be incubated in mycology for 4 weeks.
2. If Actinomyces is requested, set up a second set of anaerobic media to be incubated for 7 days before opening jar.
3. If Nocardia or Actinomyces is suggested on Gram stain, set up a second set of anaerobic media to be incubated for 7 days before opening jar and send BA and CHOC plates to Mycology after 48 hours incubation.

Rectal Abscess

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours

Bartholin's Abscess Swab/Aspirate

a) Direct Examination: Gram stain. - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Martin –Lewis Agar (ML)	CO_2, 35°C x 72 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

TISSUES, PROSTHETIC DEVICES, AND AUTOPSY SPECIMENS

Tissues/Biopsies (other than skin or transplant tissues)

Processing of Specimen:

1. When sample size allows and a fungal culture is requested, macerate the tissue using scalpels before adding to stomacher. Small pieces of tissue should be inserted into each of the fungal plates. If small sample is received then macerate the tissue using only a stomacher. Tissue around bone specimen should be removed and macerate. Dry bone can be inoculated directly into Fastidious Anaerobic Broth and not macerated. If it is a mixture of bone and tissue, macerate the tissue as usual and submerge the bone into Fastidious Anaerobic Broth
2. If fungal stains are requested, prepare 3 slides from the macerated material: one for Gram stain, one for Calcofluor white stain and one unstained (Stored in the "extra smear" slide box).
3. Fungus culture is **NOT** set up for wound debridement tissue, joint capsules, gas gangrene tissue and necrotizing fasciitis tissue unless specifically requested.
4. Send a portion of **ALL** macerated specimens or tissues to the Public Health Laboratory (PHL) for TB **except** for wound debridement tissue, joint capsules, gas gangrene tissue, and necrotizing fasciitis tissue unless specifically requested. If viral isolation is requested, send a portion of the specimen to the Virology section for processing.

5. Inoculate the following media with the remaining sample:

Media	Incubation
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours[1]
MacConkey Agar (MAC)[1]	O_2, 35°C x 48 hours[1]
Chocolate Agar (CHOC) [1]	CO_2, 35°C x 48 hours[1]
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)[1]	O_2, 35°C x 5 days[1]
If Fungus is requested, **add**:	
Inhibitory Mold Agar (IMA)[*]	O_2, 30°C x 4 weeks
Esculin Base Medium (EBM) [*]	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)[*]	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

[*] Forward the fungal culture media to the Mycology section for incubation and work-up.

Skin Biopsies

Processing of Specimen:

1. Macerate the entire specimen with a small volume of sterile saline from the original container using scalpels.
2. Prepare 3 slides from the ground material: one for Gram stain, one for Calcofluor white stain and one unstained (stored in the "extra smear" slide box).
3. If TB has been requested, forward half the specimen to the Public Health Laboratory (PHL). If viral isolation is requested, a portion of the ground material should be given to the Virology section for processing. If parasitology is requested, forward a portion of the specimen (before macerating) to the Public Health Laboratory (PHL).

a) Direct Examination: Gram stain: Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Calcofluor white stain - Refer to Mycology Manual.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
If fungus is requested, **add**::	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide	O_2, 30°C x 4 weeks

(BHIM)*

* Forward the fungus culture media to the Mycology section for incubation and work-up.

Transplant Specimens - Bone Graft & Cadaver Fascia/Tissue/ Swab Specimens/Donor Amniotic Fluid/Membrane; Donor Corneal Ring Material

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)*	O_2, 35°C x 7 days

* A separate THIO should be inoculated for each specimen / swab received.

Prosthetic Devices (e.g. Pacemaker Wire, Dacron Graft, Prosthetic Valve, Coronary/Vascular Stents)

Please note: for Urinary, urethral, prostatic and Bile duct stents, see Bile and Bile Stent section

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

Autopsy Specimens

a) Direct Examination: Gram stain - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

1. Macerate the tissue using scalpels. Tissue around bone specimen should be removed and macerate. Dry bone can be inoculated directly into Fastidious Anaerobic Broth and not macerated.
2. Prepare 2 slides from the macerated material: one for a Gram stain and a second unstained. (Stored in the "extra smear" slide box).
3. A portion of all autopsy lung tissue (except newborns) is to be sent to the Public Health Lab (PHL) for *Legionella* detection and TB culture.
4. Inoculate the following media with the remaining sample:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Colistin Nalidixic Acid Agar (CAN)	O_2, 35°C x 48 hours
For all lung tissue or if fungal culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks

* Forward the fungus culture media to the Mycology section for incubation and work-up.

CATHETER

Intravascular Catheter Tips

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35^oC x 48 hours

Roll the segment back and forth 4 times across the surface of the BA using sterile forceps.

Peritoneal Dialysis Catheter/Canula

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)	O_2, 35^oC x 5 days

BILE SPECIMENS

Bile and Bile Stents (including urinary, urethral, prostatic stents)

Inoculate one drop of specimen onto each media and smear. If stent is received, "wash" the specimen in 0.5 ml of sterile saline and inoculate the media with the solution.

a) Direct Examination: Gram stain – Examine for the presence of pus cells and organisms.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
If anaerobic culture is requested or bile is collected by PTC, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

MISCELLANEOUS FLUID SPECIMENS

Breast Milk – Clinical samples

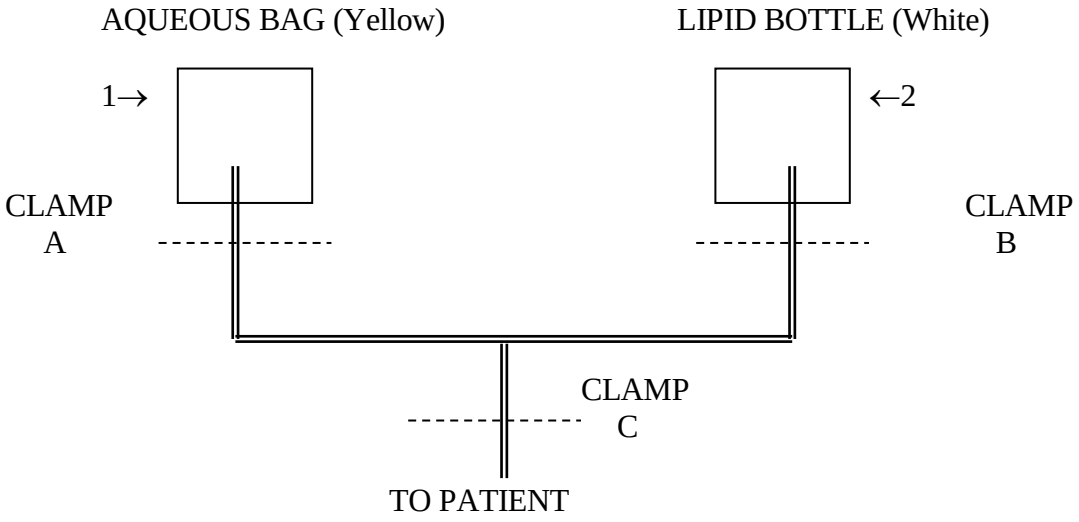
a) Direct Examination: Not required

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

Total Parental Nutrition (TPN)

1. Determine if tubings are clipped at positions A, B & C at bedside (See diagram below). If not, clip at these positions and note on work card.
2. Using aseptic technique, remove ~ 1 mL each from **1** & **2** and culture as outlined below.
3. Save the entire TPN set up for Infection Control.



b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks
IMA with sterile olive oil overlay (olive oil is stored in media room)*	O_2, 30°C x 1 week

*Forward these plates to the Mycology section for incubation and work-up.

EAR SPECIMENS

Ear Swab

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. Calcofluor white stain (If fungus is requested). - Refer to Mycology Manual.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks

* Forward the fungal culture media to the Mycology section for incubation and work-up.

Tympanocentesis Fluid

See *Other Sterile Fluids – Amniotic, Pleural (Thoracentesis/Empyema), Peritoneal (Ascites), Synovial (Joint), Pericardial, Tympanocentesis, Intraocular, Hydrocele Fluids etc*

EYE SPECIMENS

Eye / Conjunctival / Lid Swabs

Note: If pre-inoculated culture plates are received, these should be incubated as listed below. No Gram stain will be performed.

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
For all neonates ≤1 week of age, or if *N. gonorrhoeae* is requested, **add**:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

Eye / Corneal Scrapings

Note: If pre-inoculated plates are received and no smear or additional specimen is received, direct smear stains will not be performed. Result in the "TEST" field in the LIS as "No smear received, test not performed."

a) Direct Examination: Gram stain – Examine for the presence of pus cells and organisms. Calcofluor white stain (if two smears are provided). Refer to Mycology Manual.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 4 days
Chocolate Agar (CHOC)	CO_2, 35°C x 4 days
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks

*Forward the fungal culture media to the Mycology section for incubation and workup.

Intraocular Aspirates

See *Other Sterile Fluids – Amniotic, Pleural (Thoracentesis/Empyema), Peritoneal (Ascites), Synovial (Joint), Pericardial, Tympanocentesis, Intraocular, Hydrocele Fluids etc*

Lacrimal (Tear Duct) Stone / Secretions

a) Direct examination: Crush specimen on a glass slide to obtain a thin smear for Gram stain.

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a) Culture: Crush specimen using a sterile wood applicator stick or urine loop before planting onto the following media:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)[1]	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[1]	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

[1]If Actinomyces is suggested on direct Gram stain, set up a second set of anaerobic media to be incubated for 7 days before opening jar.

FACIAL SPECIMENS

Facial Swabs

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.
Calcofluor white stain (If fungus is requested).

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
If Actinomyces is requested or suggested on Gram stain or an anaerobic swab collected or thick pus is received, **add**:	
Fastidious Anaerobic Agar (BRUC)[1]	AnO_2, 35°C x 7 days
Kanamycin/Vancomycin (KV) [1]	AnO_2, 35°C x 7 days
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 days
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1]If Actinomyces is requested or suggested on direct Gram stain, set up a second set of anaerobic media to be incubated for 7 days before opening jar.

*Forward the fungal culture media to the Mycology section for incubation and work-up.

Record of Edited Revisions

Manual Section Name: **Specimen Processing Manual**

Page Number / Item	**Date of Revision**	**Signature of Approval**
Faces/Rectal Swab C&S – *Pleisiomonas* and *Aeromonas* added	April 10, 2006	Dr. T. Mazzulli
CIN agar for *Yersinia* – changed to 48 hour incubation	April 10, 2006	Dr. T. Mazzulli
Annual Review	April 10, 2006	Dr. T. Mazzulli
CSF processing – added cytospin rpm and time	August 2, 2006	Dr. T. Mazzulli
Annual Review	April 10, 2007	Dr. T. Mazzulli
BAL smear – change smear made from spun sediment before reconstituting specimen for planting	June 29, 2007	Dr. T. Mazzulli
New Mycology planting media table	June29, 2007	Dr. T. Mazzulli
MRSA screening media change	June29, 2007	Dr. T. Mazzulli
Annual Review	June 23, 2008	Dr. T. Mazzulli
Sterile fluids incubation time – changed to 5 days	July 27, 2009	Dr. T. Mazzulli
Miscellaneous Bench specimens added	July 27, 2009	Dr. T. Mazzuli
Annual Review	July 27, 2009	Dr. T. Mazzulli
Added Chocolate Agar to GC cultures	April 5, 2010	Dr. T. Mazzulli
Annual Review	April 5, 2010	Dr. T. Mazzulli
Added 0.01uL loop to suprapubic urine and removed BRUC	June 18, 2010	Dr. T. Mazzulli
Tissues, macerate with scalpels only	June 18, 2010	Dr. T. Mazzulli
Annual Review	May 31, 2011	Dr. T. Mazzulli
BAL change to planting bacteriology culture with calibrated loop	November 7, 2011	Dr. T. Mazzulli
Moved Bronchoscopy Aspirates/Washings to Sputum section	November 7, 2011	Dr. T. Mazzulli
Annual Review	May 31, 2012	Dr. T. Mazzulli
Modified planting volume into BHI broth for VRE/MRSA	August 28, 2012	Dr. T. Mazzulli
Modified Sterile Specimens incubation to 48 hours for aerobic plates	September 24, 2012	Dr. T. Mazzulli
Added ESwab handling	September 24, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli

Page Number / Item	**Date of Revision**	**Signature of Approval**
Changed Seminal fluid inoculation to CHOC and ML by drops	April 16, 2014	Dr. T. Mazzulli
Replace THIO with FA and FN bottles for Peritoneal/Ascites Fluid	April 16, 2014	Dr. T. Mazzulli
Annual Review	April 16, 2014	Dr. T. Mazzulli
Enterococcus agar changed to BVRE in dark in C&S Added *CRE Screen* Added SL solution procedure under BAL/Sputum section	November 12, 2014	Dr. T. Mazzulli
ChromID CPS4/CNA bi-plate for urine culture Tissue planting procedure	March 8, 2015	Dr. T. Mazzulli
Addition of link in urine section to Urine Hold procedure	April 30, 2015	Dr. T. Mazzulli
Updated Bloods, Platelets, & Other Transfusion Products processing procedure for direct examination and culture , (page 39)	May 26, 2015	Dr. T. Mazzulli
GBS section inserted instructions for processing subcultures. Fixed formatting and TOC headers for eye specimens	August 18, 2015	Dr. T. Mazzulli
GBS updated instructions: -specified to load in **BLUE** palette(s) labelled 'BLU URISTRAW' -removed step to ensure tiles are at the bottom of tubes -wasp program changed to all capital letter (GBS-CNA) -Added GBS processing instructions	September 4, 2015	Dr. T. Mazzulli
Updated GBS procedure Technologist loads GBS subs	September 9, 2015	Dr. T. Mazzulli
Added STAT AFB by Fluorochrome stain method p.31	October 20, 2015	Dr. T. Mazzulli
Annual Reivew	May 26, 2016	Dr. T. Mazzulli
Updated MSH logo in header Removed from fungal smears/culture processing: For special requests, send skin, hair and nail specimens to mycology for processing. – all sent to PHOL	July 4, 2016	Dr. T. Mazzulli
Streaking pattern for bronchial brush added.	July 16, 2016	Dr. T. Mazzulli
CIN added to *Plesiomonas* set up	January 4, 2017	Dr. T. Mazzulli
Asymptomatic urine hold study 68 link removed. Study 68 removed and incorporated into procedure manual.	January 31, 2017	Dr. T. Mazzulli
Reviewed BAL and Sputum sections. Updated SL Liquefying solutions to use transfer device. Removed	July 19, 2017	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
EDM solution.		
Updated positive Blood Culture subculture by WASP	December 5, 2017	Dr. T. Mazzulli
Updated urine hold procedure.	January 25, 2018	Dr. T. Mazzulli
Updated urine hold procedure.	March 6, 2018	Dr. T. Mazzulli
Addition of secondary specimen labeling. All aliquoting procedures transferred and summarized in introduction.	June 13, 2018	Dr. T. Mazzulli
Annual Review Addition of specimen safe/ethical handling in introduction.	September 13, 2018	Dr. T. Mazzulli
Enterics update to WASPlab procedure	February 5, 2019	Dr. T. Mazzulli
General Processing Instructions added.	March 15, 2019	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
p.34 Mycology – removed grinding and stomacher use for tissues processed for fungi. Tissue to be (only) gently minced to maintain fungi viability.	January 6, 2020	Jessica Bourke
p.56 Blood, Platelets, & Other Transfusion Products – addition of detailed procedure for processing bags.	January 6, 2020	Jessica Bourke
Included Stents as example in prosthetic device, added note to process bile stents per bile procedure	March 19, 2021	Wayne Chiu
Minor formatting changes	April 11, 2021	Jessica Bourke
Added info regarding urinary/urethral/prostatic vs coronary/vascular stents	April 19, 2021	Wayne Chiu
Added comment for heparinized bone marrow	May 14, 2021	Wayne Chiu
Added note regarding BVRE for flag VDE	June 7, 2021	Wayne Chiu
Fixed subculture volume for BC+ tube	July 8, 2021	Wayne Chiu
Clarified agar name for ESBL/CPE/Resistant pseudo screen culture	Nov 16, 2021	Wayne Chiu
For manual processing of blood culture bottles, ensure BCBC media is check\marked under R	May 13, 2022	Wayne Chiu
Updated bone marrow section with SPS tube	June 15, 2022	Wayne Chiu
Added Alternate SPS Blood Culture Protocol for Dimorphic Fungi	July 15, 2022	Qin Liu
Added note to CSF section – unspun sample required for	Oct 4, 2022	Wayne Chiu

biofire Removed isolator 10 tube for blood, discontinued by vendor		
Pg.44 process bronchial washing from ICU/Donor as BAL and add the following comment: }BRONC	August 9, 2023	Qin Liu