PURPOSE: A guide to the processing of specimens submitted for bacterial culture for the following samples:

1. Blood Culture:
   a. Receiving Blood Culture bottles
   b. Positive Blood Culture
   c. Blood Culture received >24 hour
2. Blood Product Culture
3. Bacterial Vaginosis Screen
4. Catheter Tip Culture
5. CSF Culture
6. Deep Wound Culture
7. Ear Culture
8. Eye Culture:
   a. Superficial Eye
   b. Deep Eye
9. Genital Culture
   a. Lower Genital Tract
   b. Upper Genital Tract
10. Gonorrhoeae Culture
11. GBS Screen
12. IUD Culture
13. MRSA Screen
14. MRO Screen
15. Nose Culture
16. Oral Culture
17. Sputum/ETT/Bronchial Wash Culture
18. Sterile Fluid (not CSF/Blood Cultures):
    a. Sterile Fluid received in sterile container
    b. Sterile Fluid received in blood culture bottles (<24 hours after collection)
    c. Sterile Fluid received in blood culture bottles (>24 hours after collection)
19. Superficial Wound Culture
20. Throat Culture
21. Urine Culture
22. VRE Screen
23. Water testing – Colilert 18
24. Water testing – HPC SimPlate
25. Wet Prep

REAGENTS and/or MEDIA:

Blood agar (BA)
MacConkey agar (MAC)
Chocolate agar (CHO)
Brucella agar (BRU)
Laked blood and KV (KV)
Thayer Martin agar (TM)
LIM broth (LIM)
Thioglycollate broth (THIO)
Sabouraud agar (SAB)
Colistin-nalidixic agar (CNA)
Denim Blue agar (DEN)
Colorex VRE (VRE)
Uri*Select* 4 agar (URI)
StrepB *Select* agar (GBS)

SUPPLIES:

- Disposable 1 µL and 10 µL loops
- Disposable needles
- Glass microscope slides
- Ringed cytology slides
- Alcohol swabs
- Sterile pipettes
- Sterile swabs
- SimPlate tubes and jars
- Colilert-18 reagent powder
- Anaerobic trays and jars
- Anaerobic indicators
- AnaeroGen packs
- Blood culture subculture vents

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to MIC60040 – Culture Media Quality Control.

PROCEDURE NOTES:

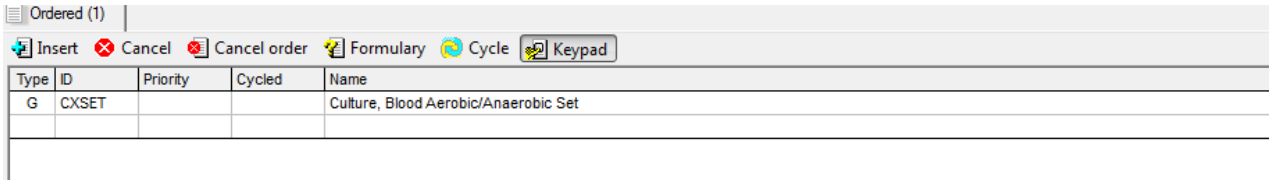
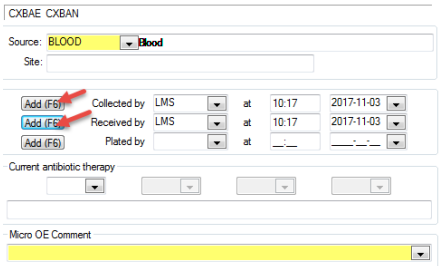
- Specimens unsuitable for culture: colostomy discharge, Foley catheter tips, gastric aspirates, lochia, vomitus.
- After processing, place specimens in the daily rack or container in the BSC.
- Discard specimens from the rack and container after 7 days.

LIMITATIONS:

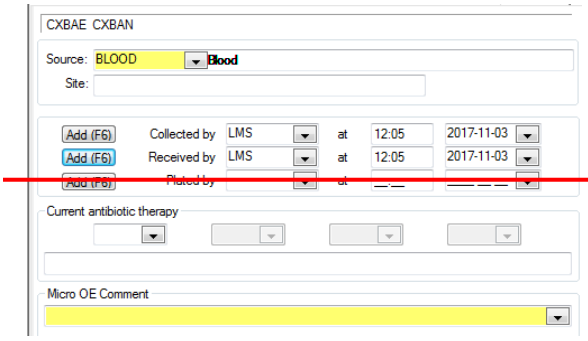
- False-positive cultures result from specimen mix-up and from contamination of media used for culture.
- False-negative cultures result from improper collection, delays in culture inoculation, inappropriate medium usage and inappropriate incubation conditions.

1. PROCEDURE INSTRUCTIONS: BLOOD CULTURE

a. Receiving Blood Culture bottles

Step	Action
1	• Blood. • Sterile fluid received in blood culture bottles.
2	Specimens should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Broken/cracked bottle. **NOTE:** If patient has been treated with antibiotics, blood culture specimens are considered irretrievable. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse. **NOTE:** Except for the above conditions, blood culture samples are not rejected regardless of delayed transport, if received frozen or if bottles are expired. Please ensure the appropriate specimen quality comments are attached to the specimen in OE and process blood culture specimen as per usual procedure.
4	Blood culture bottles need to be collected and received into SoftMic before loaded onto the BACTEC FX analyzer. It is important when receiving blood culture bottles that they are received but **NOT** plated. The instrument will not issue preliminary and final no growth reports if the specimen has been plated as it thinks the specimen has flagged positive.
5	Receiving can be done in Order Entry at the time of ordering for blood culture bottles that are being ordered by Stanton lab staff: 1. Order blood culture bottles:  2. Collect and receive bottles by selecting the **Add** button beside **Collected by** and **Received by**: 

3. Do **NOT** select on the **Add** button beside **Plated by**, leave the line blank:



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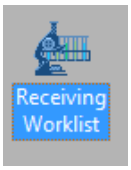
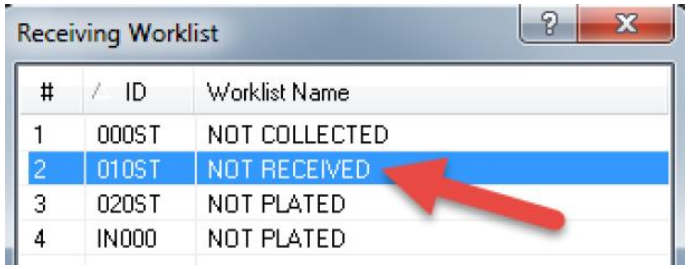
<u>Receiving of multiple bottles can be performed through the Receiving Worklist:</u>

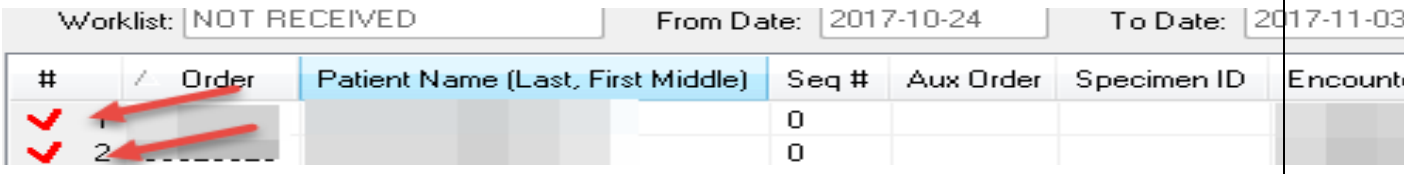
1. Double click on the **Receiving Worklist** icon on the main menu:



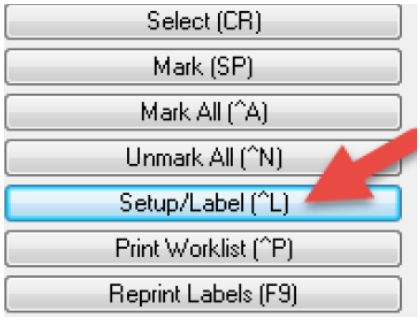
2. Double click on **Not Received**:



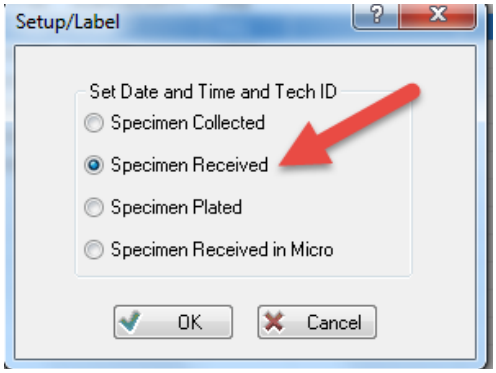
3. Scan the blood culture bottles that you want to receive. Each bottle that has been scanned will have a red check mark beside the order on the far left side:



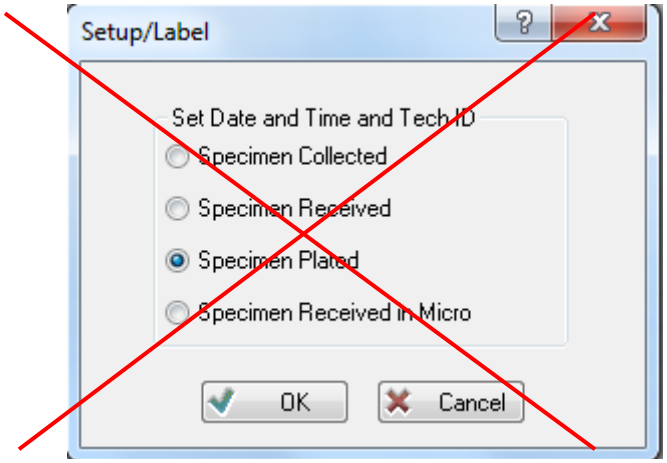
4. Select **Setup/Label** from the menu on the right hand side:



5. Ensure that **Specimen Received** is selected:

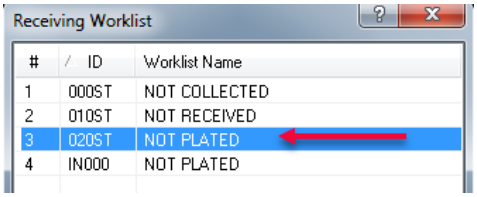
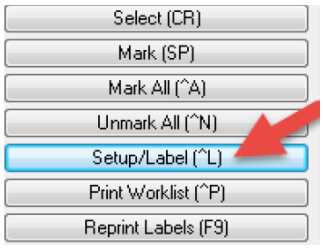
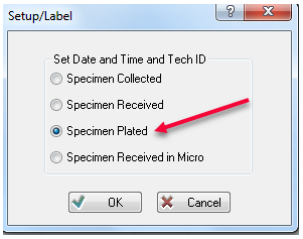


6. Ensure that **Specimen Plated** is **NOT** selected:



7. Once you have ensured that Specimen Received is selected, select the OK button to receive the specimens.
8. Load bottles onto the BACTEC FX analyzer as per MIC70300 - BACTEC FX Instrument Procedures.

b. Positive Blood Culture in BACTEC FX

Step	Action
1	Remove positive blood culture bottle(s) from the BACTEC FX. Refer to MIC70300 - BACTEC FX Instrument Procedures.
2	Generate plate labels in Order Entry: 1. Enter accession number → Micro Tab → Select F6 in the **Plated By** field → Save. Generate plate labels in Receiving Worklist: 1. Select **Receiving Worklist** icon on the main menu: 2. Select **Not Plated**:  3. Scan the blood culture bottles that you want to plate. Each bottle that has been scanned will have a red check mark beside the order on the far left side:  4. Select **Setup/Label** from the menu on the right hand side:  5. Ensure that **Specimen Plated** is selected:  6. Select OK to plate the specimens

3

Label the following media/slides

- BA-C: Blood agar
- CHO-C: Chocolate agar
- MAC-O: MacConkey agar
- BRU-2: Brucella agar
- Label the frosted end of a glass microscope slide with the accession number, patient's last name, bottle type (AE/AN/PED), specimen type (BLD).

NOTE: Please indicate which bottle is positive on ALL plates and slides.

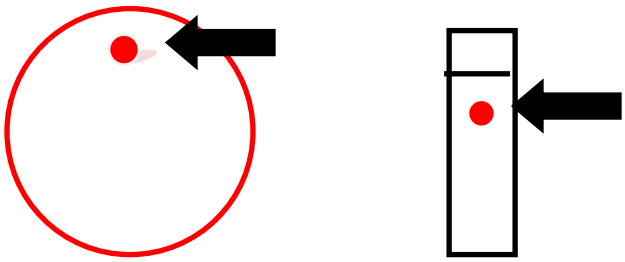
- I.e. AE for Aerobic bottle, A for Anaerobic bottle, PE for Pediatric bottle.

NOTE: Please indicate the date the bottle(s) went positive on all plates.

4

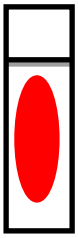
Working in the biosafety cabinet subculture the bottle(s):

- Swab the rubber septum with an alcohol pad. Insert a vent into the bottle.
- Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:



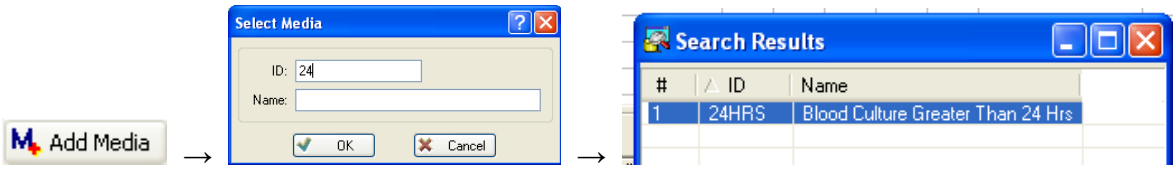
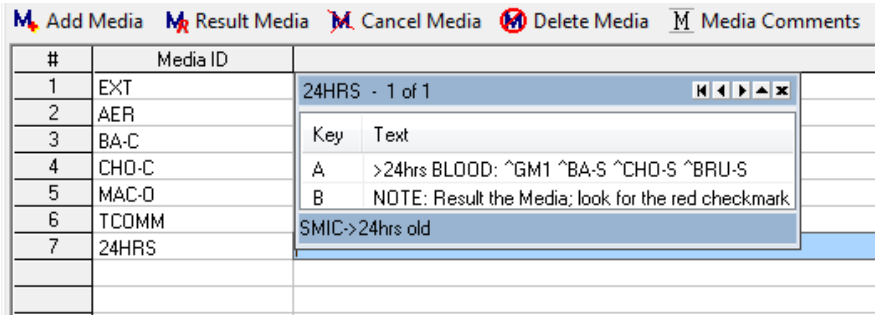
- Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet.
- Using a sterile loop, streak the plates for isolation:

- Spread the drop out on the FULL slide using the sterile loop:



5	Place MAC plate in the O_2 incubator in white tray labeled “Positive Blood Culture”.
6	Place BA and CHO plates in the CO_2 incubator in white tray labeled “Positive Blood Culture”.
7	Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
8	Gram stain slide as per Gram stain procedure MIC20115. **Positive blood culture gram stains should be read within 1 hour of processing during regular Microbiology hours of 8:00 to 20:00.**

c. Blood Culture received >24 hour

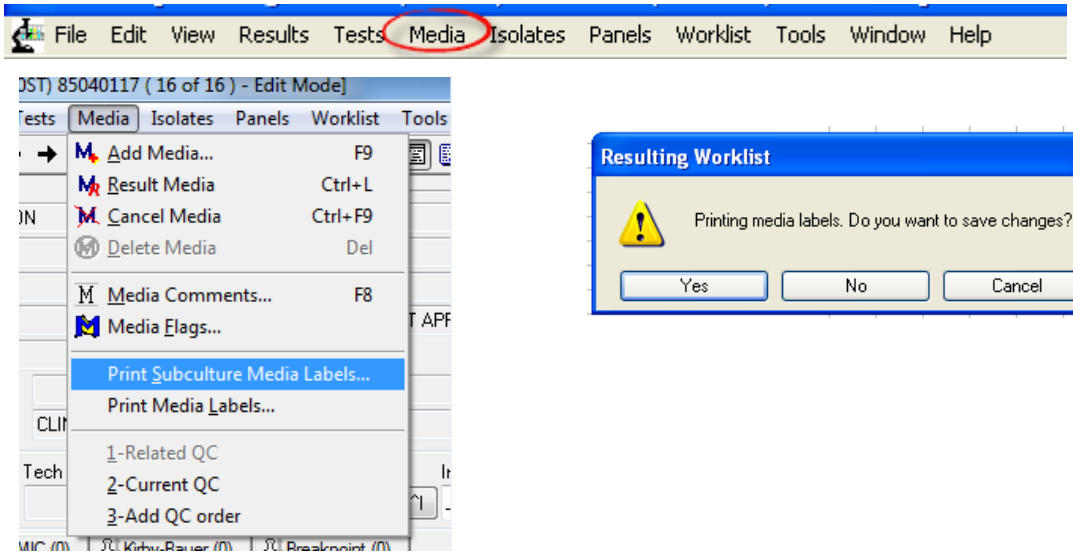
Step	Action
1	The "24" media must be ordered for each bottle received (AE, AN or PE).
2	Place the cursor in the first order in the test ID column (CXBAE) and follow the procedure to order the 24 hour media. Repeat for the second order in the test ID column (CXBAN) if applicable.
3	**In Results Entry, add the Plate Log code: "24"** 1. Select **"Add Media"** → in the 'ID" field type in **"24"** 2. Search Results screen pops up with the name of the 24HRS media ID → click OK to add it to the plate log: 
4	**Add >24 plates to the plate log:** 1. In the Media Comment line, use the keypad to select Key A to order the plates to be planted:  <u>Aerobic/ Anaerobic bottle/Pediatric bottle:</u> • GM1 • BA-S and CHO-S incubated in CO_2 incubator • BRU-S incubated anaerobically Keypad will generate appropriate plates in the lines below the 24 HRS media code.

#	Media ID	
1	EXT	
2	AER	
3	BA-C	
4	CHO-C	
5	MAC-O	
6	TCOMM	
7	24HRS	>24hrs BLOOD: -GM1 -BA-S -CHO-S -BRU-S
8	GM1	
9	BA-S	
10	CHO-S	
11	BRU-S	

5

Save changes to the plate log using the Print Subculture Media Label:

1. Select the Media menu on top of screen →
2. Scroll down and select Print Subculture Media Labels→
3. Pop-up box asks to save changes →
4. Select "Yes" to save your >24 hour media additions to the plate log (see below).



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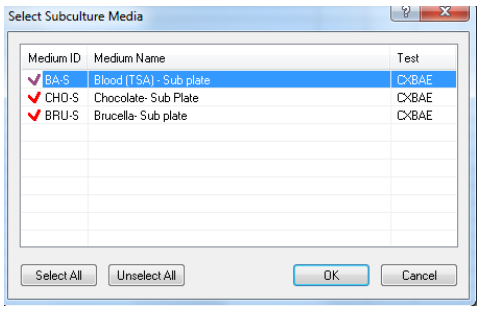
Media labels to be printed will be selected:

1. After saving changes to plate log a Select Subculture Media box generates →
   All required plates are checked off → select "OK"

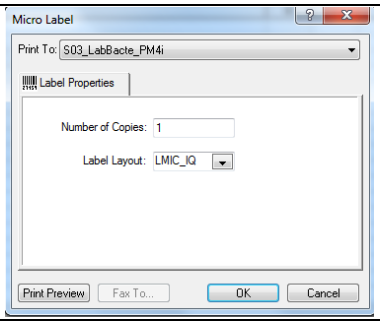


7

After selecting OK →

Micro Label box generates →

Ensure the format matches with the format in the example to the right:



8

Label the following media/slides

- BA-S: Blood agar
- CHO-S: Chocolate agar
- BRU-S: Brucella agar
- Label the frosted end of a glass microscope slide with the accession number, patient's last name, bottle type (AE/AN/PED), specimen type (BLD).

NOTE: Please indicate which bottle is > 24 hours on ALL plates and slides.

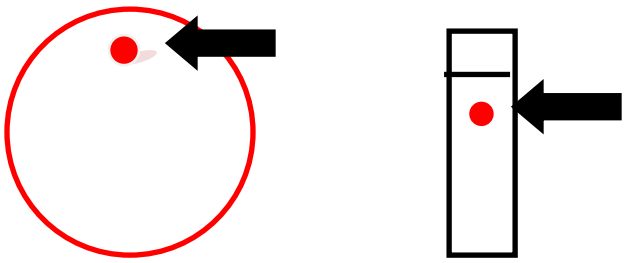
- I.e. AE for Aerobic bottle, AN for Anaerobic bottle, PE for Pediatric bottle.

NOTE: Please indicate that the plates are from a >24 hour bottle by writing "> 24 HR" on all plates.

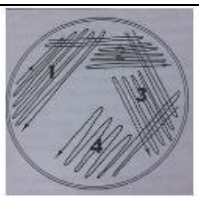
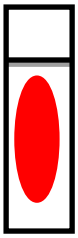
9

Working in the biosafety cabinet subculture the bottle(s):

- Swab the rubber septum with an alcohol pad. Insert a vent into the bottle.
- Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:



- Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet.
- Using a sterile loop, streak the plates for isolation:

	 • Spread the drop out on the FULL slide using the sterile loop: 
10	Load bottles onto the BACTEC FX analyzer as per MIC70300 – BACTEC FX Instrument Procedures.
11	Place BA and CHO plates in the CO_2 incubator in white tray labeled "> 24 hr Blood Culture".
12	Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
13	Gram stain slide as per Gram stain procedure MIC20115.

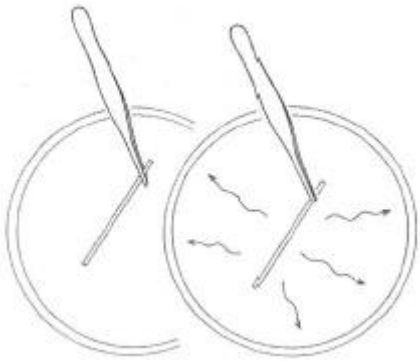
2. PROCEDURE INSTRUCTIONS: BLOOD PRODUCT CULTURE

Step	Action
1	**Blood products need to be processed as:** • Body fluid culture received in blood culture bottles (CXFBC): ➢ Source → Blood product **AND** • Body fluid culture (CXFLD): ➢ Source: → Blood product
2	Processing CXFBC: 1. 20 mL of blood product is needed for the inoculation of blood culture bottles. If sufficient volume is received, proceed to step 3. If sufficient volume is not received, aseptically inject 10 to 20 mL of Thioglycollate broth into the blood product bag and mix. 2. On the aerobic and anaerobic blood culture bottles place a mark at 10 mL above the level of the broth. Remove the caps from the blood culture bottles and clean the septum with an alcohol pad. Label bottles with LIS labels, ensuring aerobic label is placed on aerobic bottle and anaerobic label is placed on anaerobic bottle. 3. Inspect the blood product bag and tubing and determine where the material will be taken from. Use alcohol pad to clean the area where the needle will be inserted. 4. Using a butterfly needle and a vacutainer barrel, aseptically insert the needle end into the blood product bag. Using the barrel, attach a blood culture bottle and fill to the 10 mL mark. Repeat with the second bottle. Also collect a red top tube. 5. Remove the butterfly needle from the blood product and dispose of carefully into the sharps container. Place a piece of tape over the hole and place the blood product bag into a large biohazard bag and store in refrigerator until testing is complete. 6. Load bottles onto the BACTEC FX analyzer as per MIC70300 - BACTEC FX Procedures. Processing CXFLD: 1. From the red top tube collected above, use a sterile pipette to inoculate Blood agar, Chocolate agar and Brucella agar and make a gram stain with one drop of the blood product. Streak for isolated growth using a disposable inoculation needle. Streak out to cover the whole plate. 2. Place BA and CHO plates in the CO_2 incubator on “New Wound Culture” shelf. 3. Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling. 4. Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates.

3. PROCEDURE INSTRUCTIONS: BACTERIAL VAGINOSIS SCREEN

Step	Action
1	• Posterior vaginal vault or vaginal orifice. • Only performed on patient's ≥ 13 years of age. • If specimen is received on patient < 13 years of age, process as genital culture. • Refer to MIC10231 – Bacterial Vaginosis Specimen Processing Job Aid for other tests ordered on vaginal swabs.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with same collection method within 24 hours. 4. Dry swabs.
4	**Label the following media:** • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type - BV
5	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
6	Gram stain slide as per Gram-stain procedure MIC20115.

4. PROCEDURE INSTRUCTIONS: CATHETER TIP CULTURE

Step	Action
1	• Intravascular catheters including: central, CVC, Hickman, Broviac, peripheral, arterial, jugular, femoral, subclavian, umbilical, hyperalimentation, hemodialysis, port-a-cath and swan-Ganz.
2	Specimen should be refrigerated.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Foley catheter tips are not acceptable for culture – request a urine specimen. 4. Chest tube tips. 5. Abdominal drain tips. 6. Catheter tips should not be placed in saline or transport medium.
4	**Label the following media:** • BA-C: Blood agar • MAC-O: MacConkey agar
5	Using sterilized forceps, roll the segment back and forth 4 times across the surface of the Blood agar plate followed by the MacConkey plate using sterile forceps. ****If the tip is too long, cut the proximal end with sterilized scissors prior to rolling onto plates.** 
6	Place MAC plate in the O_2 incubator on “New Wound Culture” shelf.
7	Place BA plate in the CO_2 incubator on “New Wound Culture” shelf.
8	Gram stain is not performed. No slide is required.

5. PROCEDURE INSTRUCTIONS: CSF CULTURE

Step	Action
1	CSF collected from: • Central nervous system shunt fluid. • Fluid from Ommaya reservoirs. • External ventricular drainage fluid. • CSF from lumbar puncture.
2	If a delay in processing is anticipated, hold specimens at room temperature, do **NOT** refrigerate.
3	**Criteria for rejection:** 1. Insufficient volume for tests requested: contact the physician to prioritize requests. 2. Leaking specimens should be processed, but alert the physician of the possibility of contamination. 3. Improperly collected, labeled, transported or handled specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse.
4	Volume received: (Tube 2 is the usual tube for Microbiology) • **>1mL**: Centrifuge at 3500 rpm for 10 minutes (Program 2). Remove supernatant with sterile pipette and place into red top tube labeled with SUP label. Mix sediment with pipette. • **<=1mL**: Inoculate plates using a sterile pipette. **NOTE**: If sample is **NOT** centrifuged, add Specimen Quality comment **NOSPI** to state: "Sample not concentrated"
5	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • Label the frosted end of a ringed cytology slide with the accession number, patient's last name and specimen type. Clean slide with alcohol swab prior to inoculation. **NOTE: If specimen is from a shunt, THIO needs to be added**
6	**Using a STERILE pipette, dispense the fluid sediment as follows:** • 1 drop per plate. • 1 to 2 drops in the circle area of the slide. Do not spread. Allow slide to dry on the slide warmer.

7	Using a sterile loop, streak the plates for isolation: 
8	Place the remaining sample sediment and supernatant in the O_2 incubator in the rack labeled "In progress CSF".
9	Place MAC plate in the O_2 incubator in white tray labeled "CSF".
10	Place BA and CHO plates in the CO_2 incubator in white tray labeled "CSF".
11	Gram stain slide as per Gram-stain procedure MIC20115. **CSF gram stains should be read within 1 hour of processing during the regular Microbiology laboratory hours of 8:00 to 20:00.**
12	**Viral Culture ordered:** • LIS CODE: VIRO • Tube 4 is the usual tube for Viral Cultures • Transfer CSF from plastic screw top container to a glass red top tube • Freeze at -70ºC
13	**NOTE:** If there is insufficient volume for tests requested, contact the physician to prioritize requests.

6. PROCEDURE INSTRUCTIONS: DEEP WOUND/MISCELLANEOUS ASPIRATE

Step	Action
1	• Swab. • Aspirate/drainage/pus received in sterile container.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs. 4. Specimens for culture submitted in container with formalin. 5. Insufficient volume for tests requested: contact the physician to prioritize requests.
4	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • BRU-2: Brucella agar • KV-2: Laked blood, Kanamycin Vancomycin agar • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA and CHO plates in the CO_2 incubator on "New Wound Culture" shelf.
10	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
11	Gram stain slide as per Gram stain procedure MIC20115.

7. PROCEDURE INSTRUCTIONS: EAR CULTURE

Step	Action
1	• External auditory canal (outer ear). • Otitis media discharge swabbed from external auditory canal. **NOTE:** Typanocentesis fluid should be ordered as a body fluid culture.
2	Specimen should be stored at room temperature. If transport is > 2 hours, swabs should be refrigerated.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
4	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA and CHO plates in the CO_2 incubator on "New Wound Culture" shelf.
10	Gram stain slide as per Gram stain procedure MIC20115.

8. PROCEDURE INSTRUCTIONS: EYE CULTURE

a. Superficial eye: conjunctiva, superficial corneal specimens

Step	Action
1	Specimen should be stored at room temperature.
2	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
3	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
4	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
5	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
6	Using a sterile loop, streak the plates for isolation: 
7	Place BA and CHO plates in the CO_2 incubator on "New Wound Culture" shelf.
8	Gram stain slide as per Gram stain procedure MIC20115.

b. Deep Eye: corneal scrapings, aqueous/vitreous fluid, keratitis

Step	Action
1	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • BRU-2: Brucella agar • KV-2: Laked blood, Kanamycin Vancomycin agar • THIO2: Thioglycollate broth • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
2	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
3	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
4	Using a sterile loop, streak the plates for isolation: 
5	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
6	Place BA and CHO plates in the CO_2 incubator on "New Wound Culture" shelf.
7	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
8	Label THIO with Day 2 date and Day 5 date. Place THIO broth in THIO rack in O_2 incubator in "Day 2" row. **NOTE:** If the clinical information provided indicates canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, label broth with Day 10 date.
9	Gram stain slide as per Gram-stain procedure MIC20115. **Deep eye gram stains should be read within 1 hour of processing during the regular Microbiology laboratory hours of 8:00 to 20:00.**
10	Corneal scrapings are collected at patient's bedside by ophthalmologist. Give plates to physician as requested.

9. PROCEDURE INSTRUCTIONS: GENITAL CULTURE (Not BV or Gonorrhoeae)

a. Lower Genital Tract

Step	Action
1	• Vaginal vault. • Vagina or vaginal orifice. • Vulva. • Labia. • Penis.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs. 4. Do not accept vaginal swabs from women >12 years of age for genital culture unless significant clinical information is provided. Refer to MIC10231. 5. Do not process vaginal swabs for yeast culture unless significant clinical information is provided. Refer to MIC10231.
4	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • TM-C: Thayer Martin agar • MAC-O: MacConkey agar • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA, CHO and TM plates in the CO_2 incubator on "New Wound Culture" shelf.
10	Gram stain slide as per Gram stain procedure MIC20115.

b. Upper Genital Tract

Step	Action
1	• Endometrial swabs, biopsies and curettings. • Placenta swabs and tissues. • Products of conception, endometrial/uterine, Cul de Sac/transvaginal, fallopian tube, tubo-ovarian swabs or aspirates.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs. 4. Improperly collected, labeled, transported or handled irretrievable specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse.
4	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • TM-C: Thayer Martin agar • MAC-O: MacConkey agar • BRU-2: Brucella agar • KV-2: Laked blood, Kanamycin Vancomycin agar • THIO2: Thioglycollate broth • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA, CHO and TM plates in the CO_2 incubator on "New Wound Culture" shelf.

10	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
11	Label THIO with Day 2 date and Day 5 date. Place THIO broth in THIO rack in O_2 incubator in "Day 2" row.
12	Gram stain as per Gram stain procedure MIC20115.

10. PROCEDURE INSTRUCTIONS: GONORRHOEAE CULTURE

Step	Action
1	• Urethra (male specimens only). • Cervix. • Throat. • Eye. • Rectum.
2	Specimen can be stored at room temperature or refrigerated.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
4	**Label the following media/slides:** • CHO-C: Chocolate agar • TM-C: Thayer Martin agar • If the source is urethra, label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type. **Slides are only made on urethra specimens, not cervix, eye or throat.** **NOTE**: If gonorrhoeae culture is ordered on throat or eye specimens, full culture along with gonorrhoeae culture will be performed. In order entry, when ordering CXGON, if throat or eye is selected as the source, the throat culture or eye culture is automatically ordered by the LIS.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	If applicable, make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place CHO and TM plates in the CO_2 incubator on "New Wound Culture" shelf.
9	If applicable, Gram stain as per Gram stain procedure MIC20115.

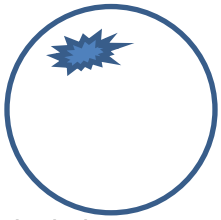
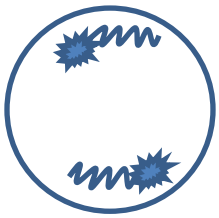
11. PROCEDURE INSTRUCTIONS: GBS SCREEN

Step	Action
1	Specimen for GBS screening in pregnancy should be collected at 35 to 37 weeks gestation.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
4	**Label the following media:** • LIM-C: LIM broth • GBS-O: StrepB *Select* agar ➢ Attach the GBS-O label to the clip on the front of the BSC.
5	Break the swab off into the LIM broth. Recap loosely.
6	**Incubate the media as follows:** • LIM Broth: CO_2 incubator ➢ This is done by the evening technologist before 20:00.
7	**After 18-24hr incubation:** • Remove the required number of StrepB *Select* agar plates from the refrigerator and bring to room temperature. • Label the GBS-O plates with the labels clipped to the BSC. • Remove LIM broth from incubator and subculture to the GBS-O plates: ➢ Saturate a sterile swab in the broth and rotate against the wall of the tube above the liquid to remove excess inoculum and swab the first quadrant of the agar. ➢ Streak for isolated growth using a disposable inoculation needle. ➢ Streak out to cover the whole plate. 
8	Incubate plate in O_2 incubator at 35° for 24 hours in the “New Urine Culture” rack.

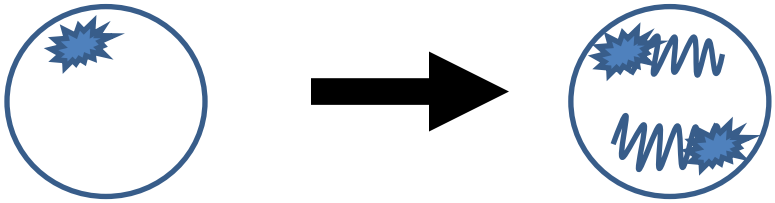
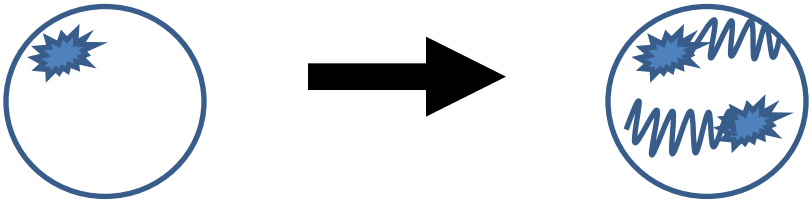
12. PROCEDURE INSTRUCTIONS: IUD CULTURE

Step	Action
1	Specimen should be refrigerated.
2	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition.
3	**Label the following media:** • THIO2: Thioglycollate broth • BRU-2: Brucella agar
4	Add a full tube of thioglycollate broth (not the labelled tube) to the specimen container containing the IUD and vortex for 30 seconds.
5	Using a sterile pipette, transfer the THIO broth into a sterile conical tube (located in TB lab) and centrifuge at 3500 rpm for 10 minutes.
6	After centrifugation is complete, using a STERILE pipette, dispense the fluid sediment as follows (discard supernatant in red top tube): • 1 drop on BRU • 2 – 5 drops in labelled Thioglycollate broth
7	Using a sterile loop, streak the plate for isolation: 
8	Label THIO with Day 2 date, Day 5 date and Day 10 date. Place THIO broth in THIO rack in O_2 incubator in “Day 2” row.
9	Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
10	Gram stain is not performed. No slide is required.

13. PROCEDURE INSTRUCTIONS: MRSA SCREEN

Step	Action
NOTE: Due to incubation requirements, MRSA plates are set up at specific times: Monday – Friday: set up at 12pm and 5pm Saturday/Sundays: set up before 3pm	
1	• Bilateral nasal swab. • Bilateral groin swab. • Other: drainages, wounds, sites of catheters, tracheostomy and other skin penetrating devices.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with same collection method from same collection location within 24 hours. 4. Dry swabs.
4	**Label the following media:** • DEN-O: Denim Blue agar ➢ For MRSA screen: label half the DEN plate with the DEN-O label
5	**Inoculate Denim Blue agar:** • Inoculate the top-left corner of the Denim Blue agar from the swab, ensuring all surfaces of swab make contact with the agar:  • Streak out for isolation: 
6	Label the DEN plates with: R: (Date + 1 date) and time incubated.
7	Place DEN plate in the O_2 incubator in MRSA 12:00 pm or 5:00 pm trays.

14. PROCEDURE INSTRUCTIONS: MRO SCREEN

Step	Action
NOTE: Due to incubation requirements, MRO Screen plates are set up at specific times: Monday – Friday: set up at 12pm and 5pm Saturday/Sundays: set up before 3pm	
1	• Swab specimen from various sources.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with same collection method from same collection location within 24 hours. 4. Dry swabs.
4	**Label the following media:** • DEN-O: Denim Blue agar ➢ For MRO-MRSA screen: label half the DEN plate with the DEN-O label • VRE-O: VRE agar ➢ For MRO-VRE screen: label half the VRE plate with the VRE-O label
5	**Inoculate Denim Blue agar:** • Inoculate the top-left corner of the Denim Blue agar from the swab, ensuring all surfaces of swab make contact with the agar and streak for isolation:  **Inoculate VRE agar:** • Inoculate the top-left corner of the VRE agar from the swab, ensuring all surfaces of swab make contact with the agar and streak for isolation: 
6	Label the DEN plates with: R: (Date + 1 date) and time incubated. Label the VRE plates with: R: (Date + 1 date and + 2 date) and time incubated.
7	Place DEN and VRE in the O_2 incubator in MRO 12:00 pm or 5:00 pm trays.

15. PROCEDURE INSTRUCTIONS: NOSE CULTURE

Step	Action
1	• Nose.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
4	**Label the following media:** • BA-C: Blood agar
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Using a sterile loop, streak the plate for isolation: 
7	Place BA plate in the CO_2 incubator on “New Wound Culture” shelf.
8	Gram stain is not performed. No slide is required.

16. PROCEDURE INSTRUCTIONS: ORAL CULTURE

Step	Action
1	• Mouth. • Tongue.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.
4	**Label the following media:** • SAB-R: Sabouraud dextrose agar
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Using a sterile loop, streak the plate for isolation: 
7	Label the SAB plate with: R: (Date + 2 date).
8	Place SAB plate on wound bench (incubated at room temperature).
9	Gram stain is not performed. No slide is required.

17. PROCEDURE INSTRUCTIONS: SPUTUM/ETT/BRONCHIAL WASH CULTURE

Step	Action
1	• Sputum. • Endotracheal aspirate. • Auger suction. • Bronchial aspirates (washings). • Bronchoalveolar lavage (BAL).
2	Specimens should be refrigerated.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Swabs of sputa. 4. Duplicate specimens obtained with the same collection method within 24 hours. 5. Specimen is > 72 hours old. 6. Leaking specimens. 7. Improperly collected, labeled, transported or handled bronchial aspirate (wash specimens), BAL specimens, lung aspirates and lung biopsy specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse.
4	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • Label the frosted end of a glass microscope slide with accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA and CHO plates in the CO_2 incubator on "New Wound Culture" shelf.
10	Gram stain as per Gram-stain procedure MIC20115.

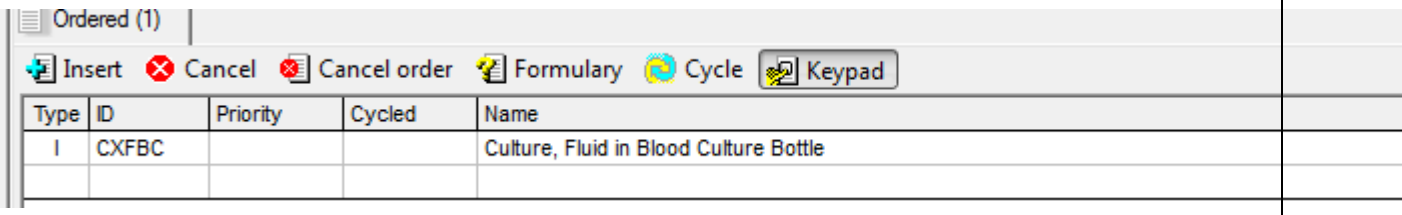
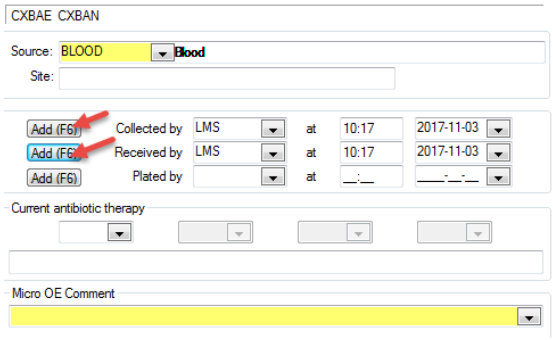
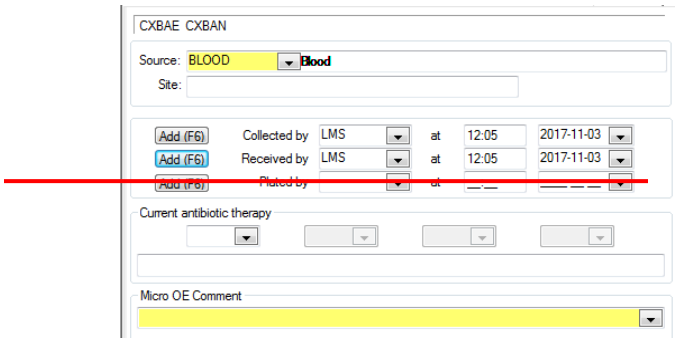
18. PROCEDURE INSTRUCTIONS: STERILE FLUID (NOT CSF/BLOOD)

a. Sterile fluid received in sterile container:

Step	Action
1	• Fluid should be collected in a sterile specimen container or tube and/or into blood culture bottles. • If fluid is received in blood culture bottles, order as Blood Culture-Fluid and process as blood culture. Refer to part b. of this section. • If swab is received, add Specimen Quality comment **SWBFL** which states: **"Swab sample may be inadequate for recovery of organisms. Interpret results with caution"**. • Refer peritoneal fluid specimens for culture to DynaLIFE. • Refer prosthetic device specimens for culture to DynaLIFE. • Refer tissue or biopsy specimens for culture to DynaLIFE.
2	If a delay in processing is anticipated, hold specimens at room temperature, do NOT refrigerate.
3	**Criteria for rejection:** 1. Insufficient volume for tests requested: contact the physician to prioritize requests. 2. Leaking specimens should be processed, but alert the physician of the possibility of contamination. 3. Specimens received in the laboratory in a syringe with the needle still attached will be rejected. In addition, a RiskPro will be filed outlining the hazard. Refer to SCM40100 - Specimen Acceptance and Rejection Policy. 4. Improperly collected, labeled, transported or handled specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse. 5. If only blood culture bottles are received, a gram stain cannot be performed.
4	Volume received: (Tube 2 is the usual tube for Microbiology) • **>1mL**: Centrifuge at 3500 rpm for 10 minutes (Program 2). Remove supernatant with sterile pipette and place into red top tube. Mix sediment with pipette. • **<=1mL**: Inoculate plates using a sterile pipette. **NOTE:** If sample is NOT centrifuged → add Specimen Quality comment **NOSPI** to state: "Sample not concentrated"
5	**Label the following media/slides:** • BA-C: Blood agar • CHO-C: Chocolate agar • MAC-O: MacConkey agar • BRU-2: Brucella agar • KV-2: Laked blood, Kanamycin Vancomycin agar • THIO2: Thioglycollate broth • Label the frosted end of a ringed cytology slide with the accession number, patient's last name and specimen type. Clean slide with alcohol swab prior to inoculation.

6	**Using a STERILE pipette, dispense the fluid sediment as follows:** • 1 drop per plate • 1 drop in the circle area of the slide. Allow slide to dry on the slide warmer • 2 – 5 drops in Thioglycollate broth
7	Using a sterile loop, streak the plates for isolation: 
8	Place the remaining sample sediment in the O_2 incubator.
9	Place MAC plate in the O_2 incubator on “New Wound Culture” shelf.
10	Place BA and CHO plates in the CO_2 incubator on “New Wound Culture” shelf.
11	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
12	Label THIO with Day 2 date and Day 5 date. Place THIO broth in THIO rack in O_2 incubator in “Day 2” row.
13	Gram stain as per Gram stain procedure MIC20115. **Fluid gram stains should be read within 1 hour of processing during the regular Microbiology laboratory hours of 8:00 to 20:00.**

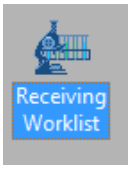
b. Sterile fluid received in Blood Culture bottle (<24 hours after collection):

Step	Action
1	Sterile body fluids received in blood culture bottles need to be collected and received into SoftMic before loaded onto the BACTEC FX analyzer. It is important when receiving sterile body fluids blood culture bottles that they are received but **NOT** plated. The instrument will not issue preliminary and final no growth reports if the specimen has been plated as it thinks the specimen has flagged positive.
2	<u>Receiving can be done in Order Entry at the time of ordering for blood culture bottles that are being ordered by Stanton lab staff.</u> 1. Order sterile body fluid received in blood culture bottles as **CXFBC**:  2. Collect and receive bottles by selecting the Add button beside **Collected by** and **Received by**:  3. Do **NOT** select the Add button beside **Plated by**, leave the line blank: 

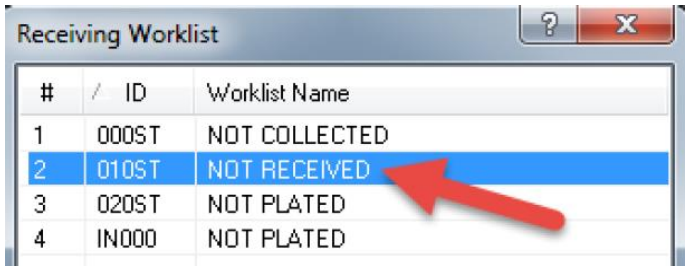
3

Receiving of multiple bottles can be performed through the Receiving Worklist.

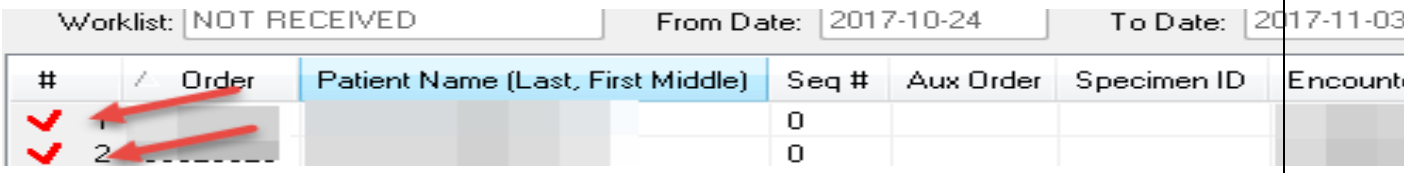
1. Double click on the **Receiving Worklist** icon on the main menu:



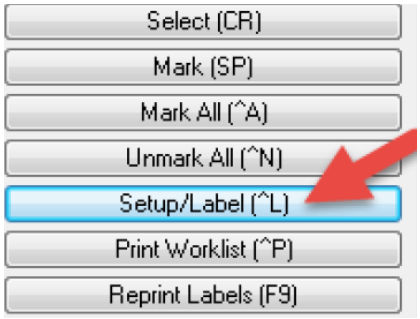
2. Double click on **Not Received**:



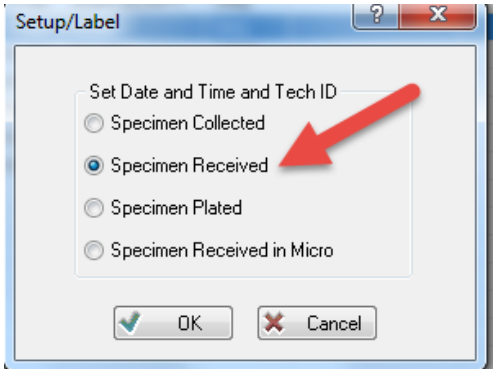
3. Scan the blood culture bottles that you want to receive. Each bottle that has been scanned will have a red check mark beside the order on the far left side:



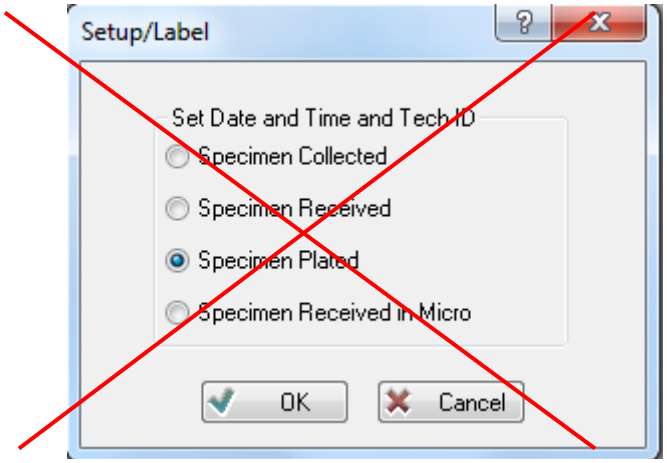
4. Select **Setup/Label** from the menu on the right hand side:



5. Ensure that **Specimen Received** is selected:

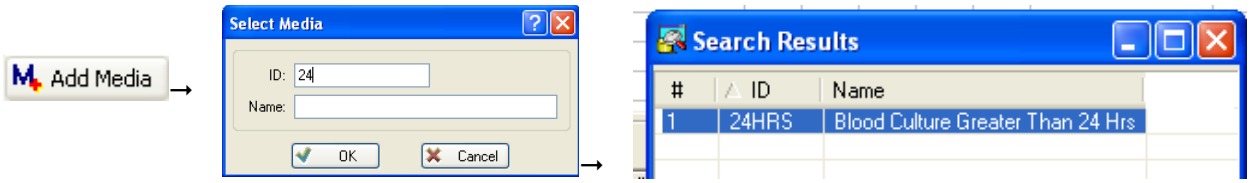
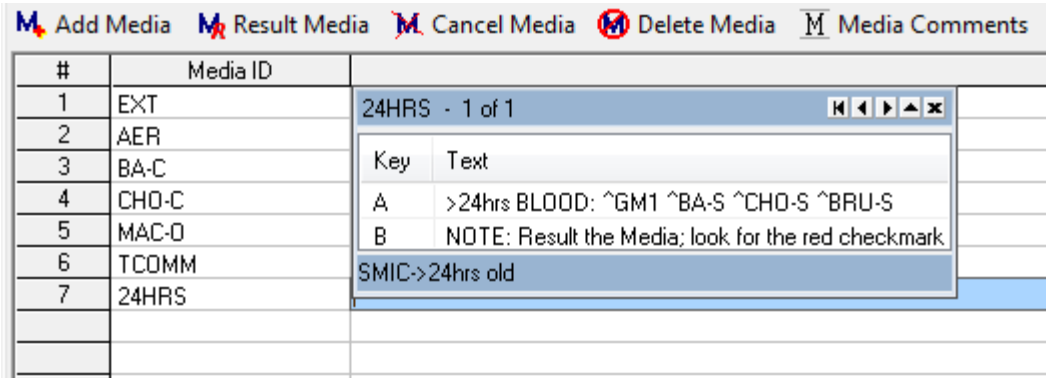
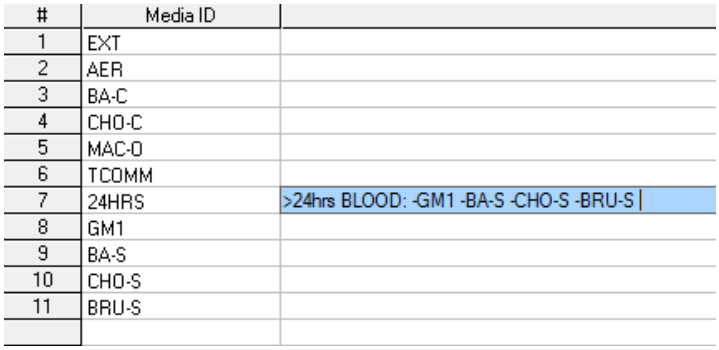


6. Ensure that Specimen Plated is NOT selected:



7. Once you have ensured that Specimen Received is selected, select the OK button to receive the specimens.
8. Load bottles onto the BACTEC FX analyzer as per MIC70300 - BACTEC FX Instrument Procedures.

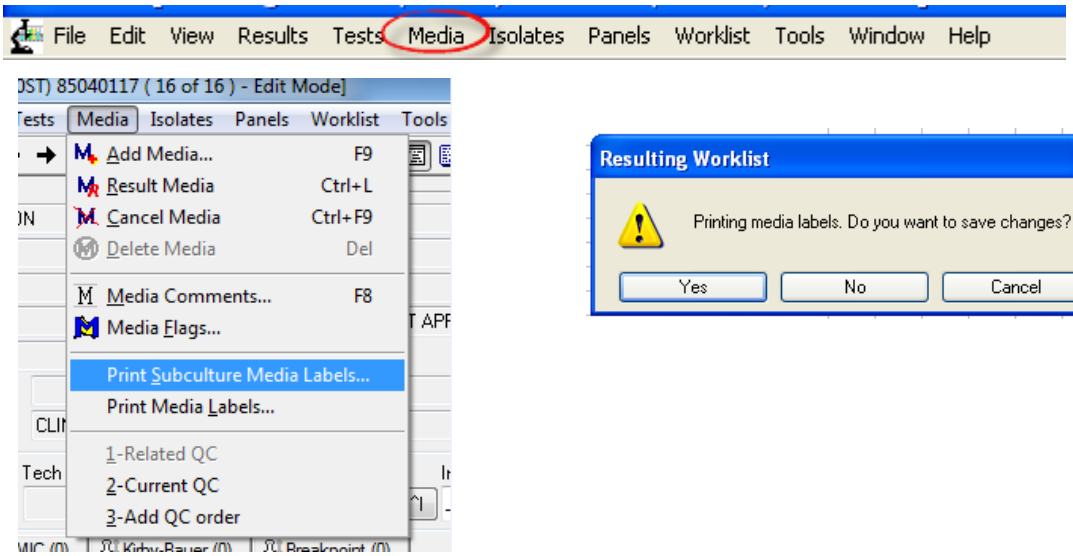
c. Sterile fluid received in Blood Culture bottle (>24 hours after collection):

Step	Action
1	**In Results Entry, add the Plate Log code: "24"** 1. Select **"Add Media"** → in the 'ID" field type in **"24"** 2. Search Results screen pops up with the name of the 24HRS media ID → click OK to add it to the plate log. 
2	**Add >24 plates to the plate log:** 1. In the Media Comment line, use the keypad to select Key A to order the plates to be planted:  <u>Aerobic/ Anaerobic bottle/Pediatric bottle:</u> • GM1 • BA-S and CHO-S incubated in CO_2 incubator • BRU-S incubated anaerobically Keypad will generate appropriate plates in the lines below the 24 HRS media code: 

3

Save changes to the plate log using the Print Subculture Media Label:

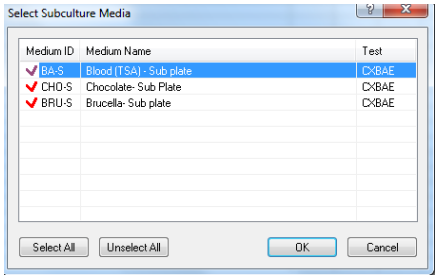
1. Select the Media menu on top of screen →
2. Scroll down and select Print Subculture Media Labels→
3. Pop-up box asks to save changes →
4. Click "Yes" to save your >24 hour media additions to the plate log (see below).



4

Media labels to be printed will be selected:

1. After saving changes to plate log a Select Subculture Media box generates →
   All required plates are checked off → select "OK"

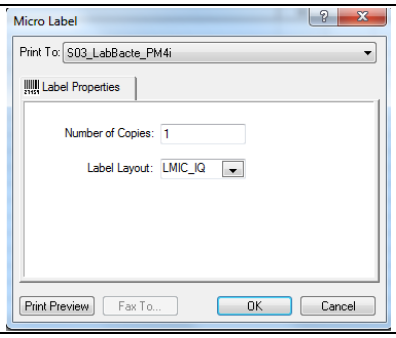


5

After selecting OK →

Micro Label box generates →

Ensure the format matches with the format in the example to the right:



6

Label the following media/slides:

- BA-S: Blood agar
- CHO-S: Chocolate agar
- BRU-S: Brucella agar
- Label the frosted end of a glass microscope slide with the accession number, patient's last name, bottle type (AE/AN/PED), specimen type (BLD).

NOTE: Please indicate which bottle is > 24 hours on ALL plates and slides.

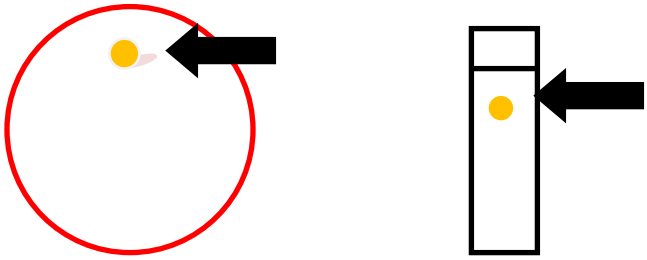
- I.e. AE for Aerobic bottle, AN for Anaerobic bottle, PE for Pediatric bottle.

NOTE: Please indicate that the plates are from a >24 hour bottle by writing "> 24 HR" on all plates.

7

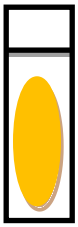
Working in the biosafety cabinet subculture the bottle(s):

- Swab the rubber septum with an alcohol pad. Insert a vent into the bottle.
- Holding the bottle horizontally, place one drop on each plate and one small drop on the slide.



- Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet.
- Using a sterile loop, streak the plates for isolation:

- Spread the drop out on the FULL slide using the sterile loop:



8	Load bottles onto the BACTEC FX analyzer as per MIC70300 - BACTEC FX Instrument Procedures.
9	Place BA and CHO plates in the CO_2 incubator on “New Wound Culture” shelf.
10	Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10220 – Microbiology Specimen Handling.
11	Gram stain as per Gram stain procedure MIC20115.

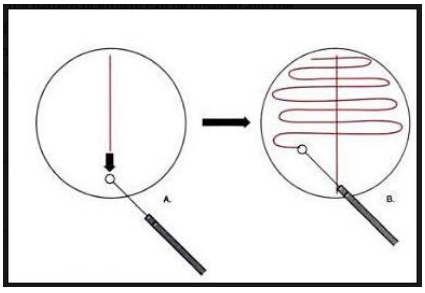
19. PROCEDURE INSTRUCTIONS: SUPERFICIAL WOUND CULTURE

Step	Action
1	1. Superficial wound specimens: ➢ Abrasion, cut, laceration, ulcer, skin diseases (impetigo, folliculitis, cellulitis), first degree burn, superficial surgical incision, etc. 2. Superficial specimens: ➢ Boils, cyst, etc. 3. Drain specimens: ➢ J-tubes, G-tubes, chest tube, abdominal, etc.
2	Specimens should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs. 4. Specimens for culture submitted in container with formalin. 5. Submission of specimens to determine *if* an infection is present should be discouraged.
4	**Label the following media:** • BA-C: Blood agar • MAC-O: MacConkey agar • Label the frosted end of a glass microscope slide with accession number, patient's last name and specimen type.
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10220 – Microbiology Specimen Handling.
7	Using a sterile loop, streak the plates for isolation: 
8	Place MAC plate in the O_2 incubator on "New Wound Culture" shelf.
9	Place BA plate in the CO_2 incubator on "New Wound Culture" shelf.
10	Gram stain as per Gram stain procedure MIC20115.

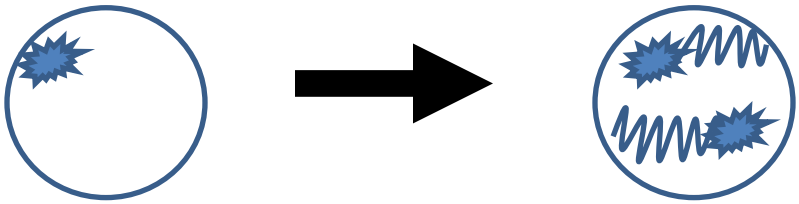
20. PROCEDURE INSTRUCTIONS: THROAT CULTURE

Step	Action
1	• Throat swab.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with same collection method within 24 hours. 4. Dry swabs.
4	**Label the following media:** • BA-2: Blood agar
5	Inoculate plates with specimen. Refer to MIC10220 – Microbiology Specimen Handling.
6	Using a sterile loop, streak the plate for isolation: 
7	Place BA plate in "For Throat Jar" rack in CO_2 incubator.
8	Before leaving in the evening, the technologist will place rack in anaerobic jar with anaerobic pouch and indicator. Label jar with date of 24 hour read.
9	Gram stain is not performed. No slide is required.

21. PROCEDURE INSTRUCTIONS: URINE CULTURE

Step	Action
1	• Fresh urine collected in sterile container. • Fresh urine collected in urine transport tube.
2	Urine in sterile container should be refrigerated. Urine in urine transport tube can be kept at room temperature or refrigerated.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with the same collection method within 24 hours. 4. Refrigerated fresh urine specimens received more than 24 hours after collection. 5. Blue top urine specimens received more than 72 hours after collection. 6. 24 hour urine collections. 7. Foley catheter tips. 8. Specimens in leaking container.
4	**Label the following media:** • UR1-O: Uri*Select* 4 agar for non-sterile urine specimens • UR2-O: Uri*Select* 4 agar for sterile urine specimens. **Highlight urine type on plate**
5	Mix specimen by swirling or gentle inversion. Dip a sterile calibrated 0.001 mL loop (green) vertically into the sample just below the surface of the urine.
6	Inoculate with the loop down the center of the plate and then cross-streak at a 90 degree angle to the inoculum: 
7	Place plate in the rack and place rack in O_2 incubator on “New Urine Culture” shelf. **NOTE:** Place plates in the incubator as soon as practical after inoculation. **NOTE:** If incubating after 6pm, plates must be placed in a rack that specifies they were incubated after 6pm to ensure adequate incubation time is reached.

22. PROCEDURE INSTRUCTIONS: VRE SCREEN

Step	Action
NOTE: Due to incubation requirements, VRE plates are set up at specific times Monday – Friday: set up at 12pm and 5pm Saturday/Sundays: set up at 2:30pm	
1	• Swab specimen. • Stool specimens.
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Duplicate specimens obtained with same collection method from same collection location within 24 hours. 4. Dry swabs. 5. Nasal and axilla swabs should not be processed for VRE. 6. For swabs not visibly soiled with fecal matter, add specimen quality comment **VRE** to state**: "No fecal matter visible on swab"**.
4	**Label the following media:** • VRE-O: VRE agar ➢ For VRE screen: label half the VRE plate with the VRE-O label
5	**Inoculate VRE agar:** • Inoculate the top-left corner of the VRE agar from the swab, ensuring all surfaces of swab make contact with the agar and streak for isolation: 
6	Label the VRE plates with: R: (Date + 1 date and + 2 date) and time incubated.
7	Place the VRE in the O_2 incubator, in VRE 12:00 pm or 5:00 pm trays.

23. PROCEDURE INSTRUCTIONS: WATER - COLILERT-18

Step	Action
1	• 100 mL of water.
2	Specimen should be refrigerated.
3	**Criteria for rejection:** 1. Received > 48 hours after collection.
4	Accession waters and generate labels. Label requisition and sample containers.
5	If HPC testing is required, it must be performed prior to the addition of the Colilert-18 reagent.
6	Pour off excess water until the volume is ~100mLs.
7	Incubate at 35°C for 1 hour – write the time of day on one of the water vessels.
8	Add Colilert-18 reagent power to each water vessel – look for a blue flash – if seen this indicates excessive chlorine and the test is invalid.
9	Shake to mix well.
10	Place bottles back in incubator.

24. PROCEDURE INSTRUCTIONS: WATER - HPC UNIT DOSE SIMPLATE

Step	Action
1	• Hot tub water.
2	Specimen should be refrigerated.
3	**Criteria for rejection:** 1. < 10 mL water received. 2. Received > 48 hours after collection.
4	Accession waters and select source. If source is HOT TUB or SPA WATER – HPC will be automatically ordered and a MacConkey plate label will generate.
5	Label requisition and sample containers.
6	Label sterile media tube (green top), SimPlate and MAC plate (where required).
7	Mix water. Use a 10 mL syringe to remove 10 mL of water from sample container. Add to green top tube and shake. Allow powder in tube to dissolve.
8	Remove SimPlate lid and pour contents of green top tube onto the center of the plate base.
9	Replace the lid and gently swirl to distribute the sample. **NOTE:** air bubbles do not interfere with test.
10	Tip the plate at a 90° angle so the excess water will drain into the absorbent pad at the bottom of the plate.
11	Invert the plate onto the plastic lid. On lid write R: (Date + 2 date) and time incubated
12	If water source is HOT TUB, perform total coliform testing as well using the Colilert-18 kit and processed as above.
13	If water is HOT TUB, aliquot an additional 1mL of water and flood a labeled MAC plate - incubate along with the SimPlate (35°C for 48hrs).
14	Place SimPlate into incubator along with MAC plate if required.

25. WET PREP

Step	Action
1	• Vaginal swab. • Cervix. • Endocervix. • Urethra (male and female).
2	Specimen should be stored at room temperature.
3	**Criteria for rejection:** 1. Specimen is > 72 hours old. Refer to MIC10231 – Bacterial Vaginosis Specimen Processing Job Aid. 2. Unlabeled/mislabeled specimen. 3. Specimen container label does not match patient identification on requisition. 4. Duplicate specimens obtained with same collection method within 24 hours. 5. Dry swabs.
4	Label the following media: • WPGS: Glass test tube
5	Place labeled glass test tube into a rack and add approximately 0.5 mL of saline.
6	Place the culture swab into the saline and mix. Place the swab transport tube in the slot behind the glass test tube.
7	Incubate in the O_2 incubator for 15 minutes.
8	Let the microbiology technologists know that wet preps have gone into the incubator.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
  Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015.
- Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	11 AUG 2013	Initial Release	A. Darrach
2.0	12 FEB 2019	Update to reflect 2 VRE and MRO samples per plate.	L. Steven
3.0	10 June 2019	Update to reflect new urine chromogenic agar	L. Steven