PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC10100 – Microbiology Specimen Processing	Policy Number: 15-146-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 15/03/2024	Next Review Date: 15/03/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 15/03/2024
Accreditation Canada Applicable Standard: NA	

GUIDING PRINCIPLE:
A guide to the processing of specimens submitted for bacterial culture for the following specimens:

PURPOSE/RATIONALE:
This standard operating procedure describes the specimen processing for microbiology specimens at Stanton Territorial Hospital.

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for microbiology culture.

REAGENTS and/or MEDIA:
- Anaerobic KV agar (KV)
- Blood agar (BA)
- Brucella agar (BRU)
- Candi*Select* agar (YST-O)
- Chocolate agar (CHO)
- Colistin-nalidixic acid agar (CNA)
- LIM broth (LIM)
- MacConkey agar (MAC)
- MRSA*Select* II agar (MRS)
- StrepB*Select* agar (GBS)
- Thayer Martin agar (TM)
- Thioglycollate broth (THIO)
- Uri*Select* 4 agar (URI)
- VRE*Select* agar (VRE)

SUPPLIES:
- Disposable 1 µL and 10 µL loops
- Disposable needles
- Glass microscope slides
- Ringed cytology slides
- Alcohol pads
- Sterile pipettes
- Sterile swabs
- Anaerobic trays and jars
- Anaerobic indicators
- AnaeroGen packs
- AnaeroPouch packs
- Blood culture subculture vents

EQUIPMENT:
- Biosafety cabinet
- 35° O_2 and 35° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:
Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:
- Ensure that appropriate hand hygiene practices be used
- 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 when there is a known or potential risk of exposure of 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. Routine Practices 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:

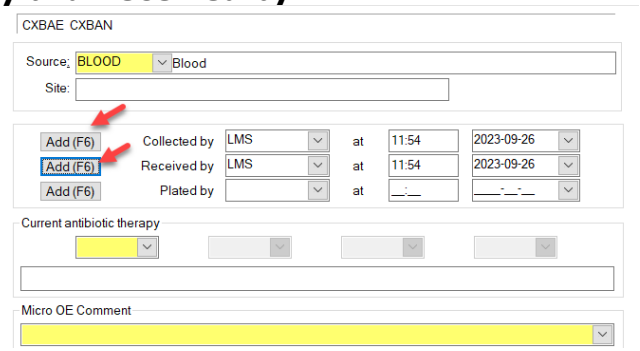
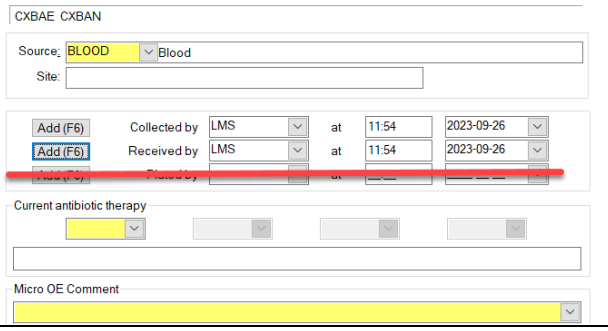
- Refer to MIC60010-Microbiology Quality Control procedure
- Refer to MIC60040-Culture Media Quality Control procedure

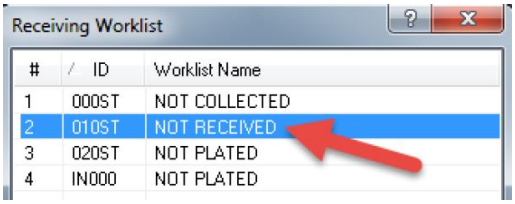
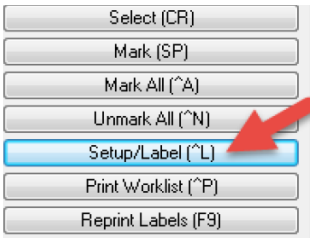
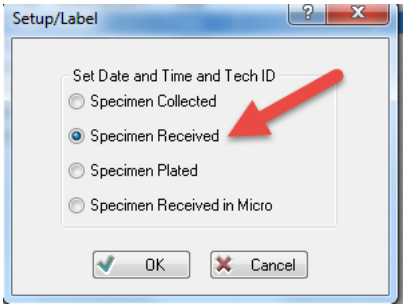
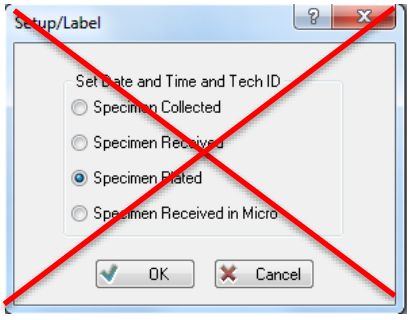
1. PROCEDURE INSTRUCTIONS: BACTERIAL VAGINOSIS SCREEN

Step	Action
1	• Posterior vaginal vault or vaginal orifice • Only performed on patients that are >13 years of age • If specimen is received on patient ≤13 years of age, process as a genital culture • Refer to MIC10110-Vaginal Swab 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	Label the following media/slides: • Label the frosted end of a glass microscope slide with the accession number, patient's last name and specimen type
5	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
6	Gram stain slide. Refer to MIC20115-Gram Stain.

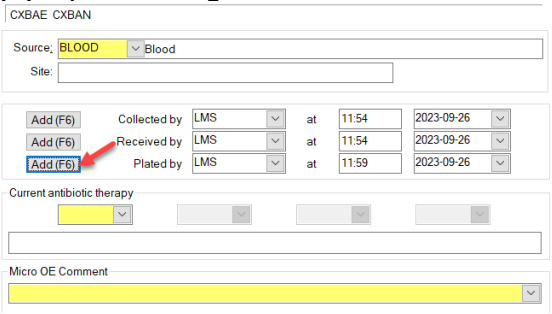
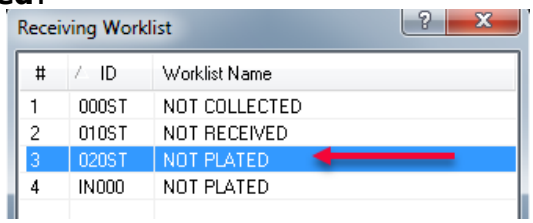
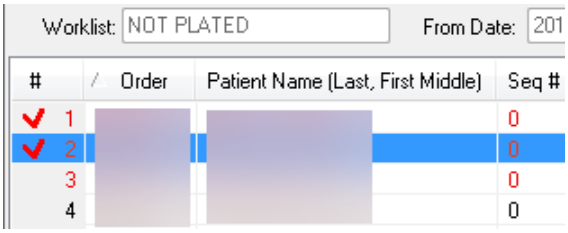
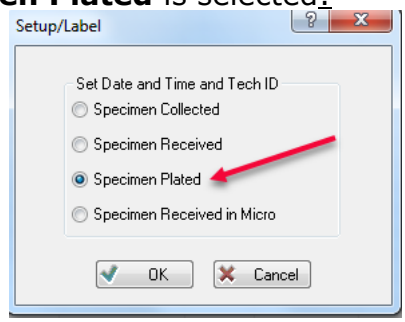
2. PROCEDURE INSTRUCTIONS: BLOOD CULTURE

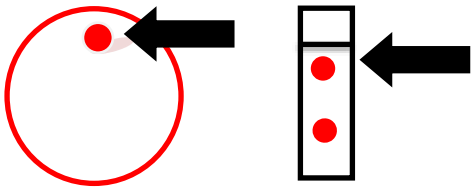
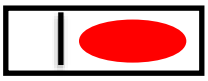
a. Receiving Blood Culture bottles

Step	Action
1	• Blood • Sterile fluid received in blood culture bottle
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. Broken/cracked bottle **NOTE:** If patient has been treated with antibiotics, blood culture specimens are considered irretrievable. SCM40110-Waiver of Responsibility form needs to be filled out by the responsible nurse **NOTE:** Except for the above conditions, blood culture specimens are not rejected regardless of delayed transport, if received frozen or if bottles are expired. Ensure the appropriate specimen quality comments are attached to the specimen in ORDER ENTRY and process blood culture specimen
4	Blood culture bottles need to be ordered, collected and received into SoftMic before loaded onto the BACTEC FX analyzer. However, it is important that bottles are received but **NOT** plated. The instrument will not issue preliminary and final no growth reports if the specimen has been plated in the LIS.
5	Receiving can be performed in Order Entry: 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 the **Add** button beside **Plated by:** 

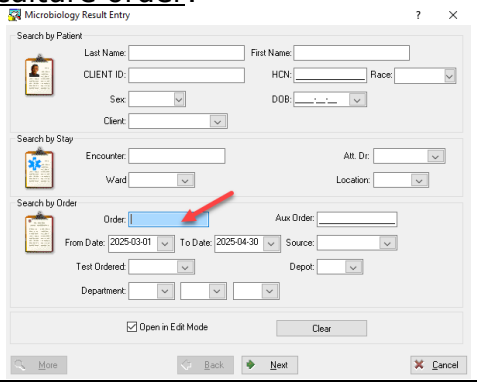
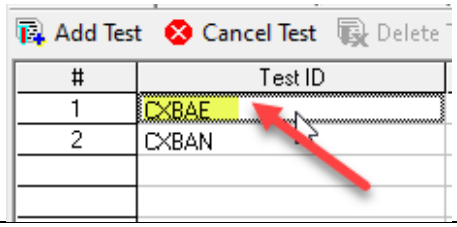
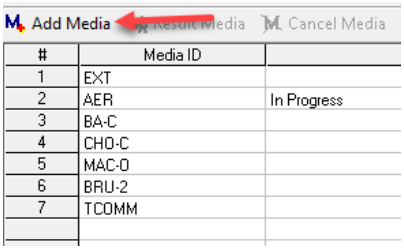
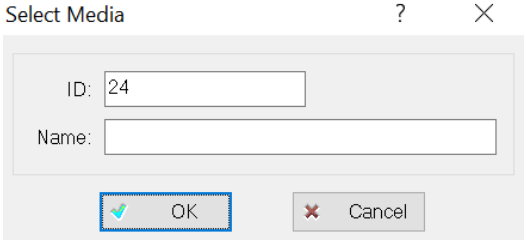
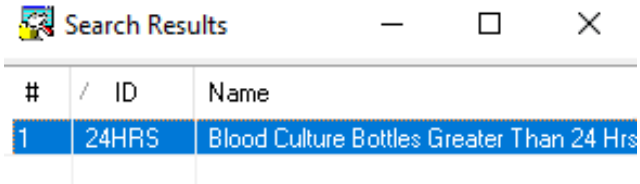
6	Receiving of multiple bottles can be performed in the Receiving Worklist: 1. Select **Receiving Worklist** icon on the main menu 2. Select **Not Received:**  3. Scan the blood culture bottles that you want to receive. Each bottle that has been scanned will have a red check mark beside the order on the 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 MIC71000-BACTEC FX Instrument

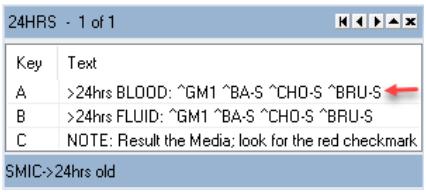
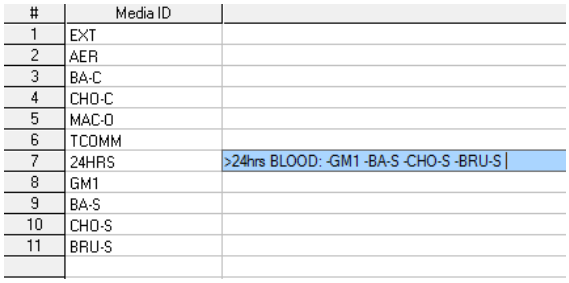
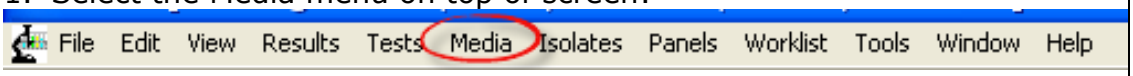
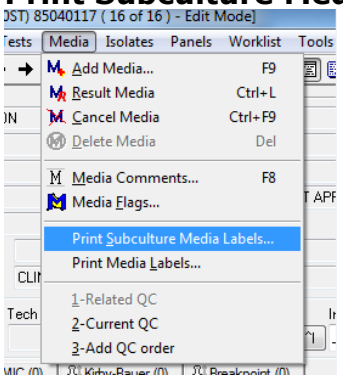
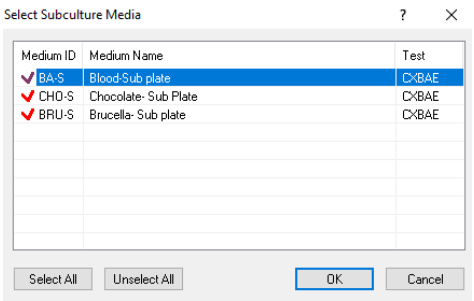
b. Positive Blood Culture in BACTEC FX

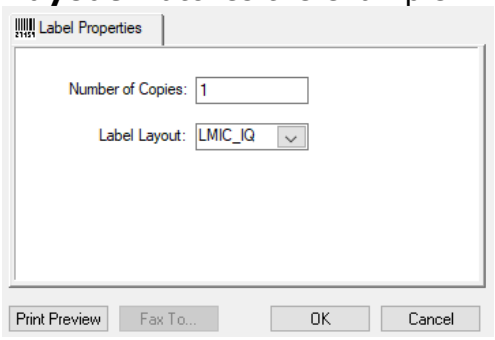
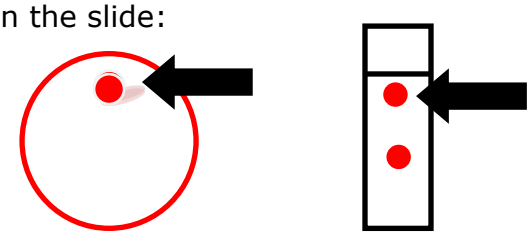
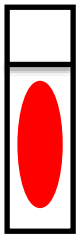
Step	Action
1	Remove positive blood culture bottle(s) from the BACTEC FX. Refer to MIC71000-BACTEC FX Instrument.
2	<u>Plating can be performed in Order Entry</u>: 1. Enter accession number 2. Select the Micro Tab 3. Plate the bottle(s) by selecting the **Add** button beside **Plated by:**  <u>Plating can be performed in Receiving Worklist</u>: 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 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) • Clean slide with alcohol pad prior to inoculation **NOTE:** Indicate which bottle is positive on ALL plates and slides **NOTE:** Indicate the date the bottle(s) went positive on all plates
4	Working in the biosafety cabinet subculture the bottle(s): 1. Swab the rubber septum with an alcohol pad and insert a vent 2. Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:  3. Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet 4. Using a sterile loop, streak the plates for isolation 5. Spread the drops out on the FULL slide using the sterile loop: 
5	Place MAC plate on the "BLOOD CULTURES" shelf in the O_2 incubator.
6	Place BA and CHO plates in the lower CO_2 incubator on the "New + Blood Cultures" shelf.
7	Place BRU plate in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO_2" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
8	Gram stain slide. Refer to MIC20115-Gram Stain. **NOTE:** Positive blood culture gram stains should be read within 1 hour of processing during regular Microbiology hours

c. Blood Culture received >24 hr

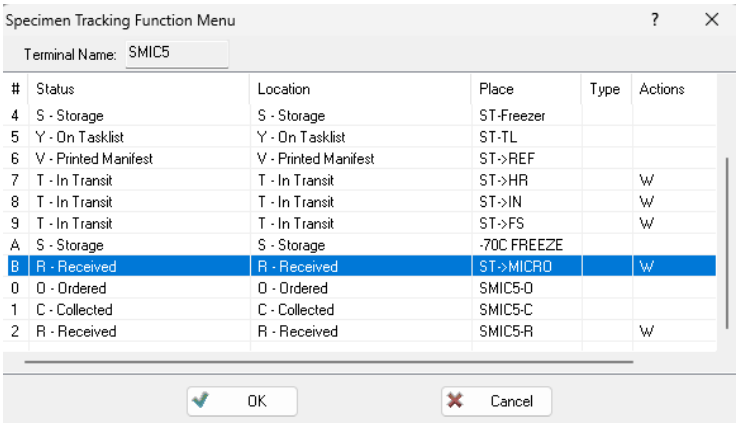
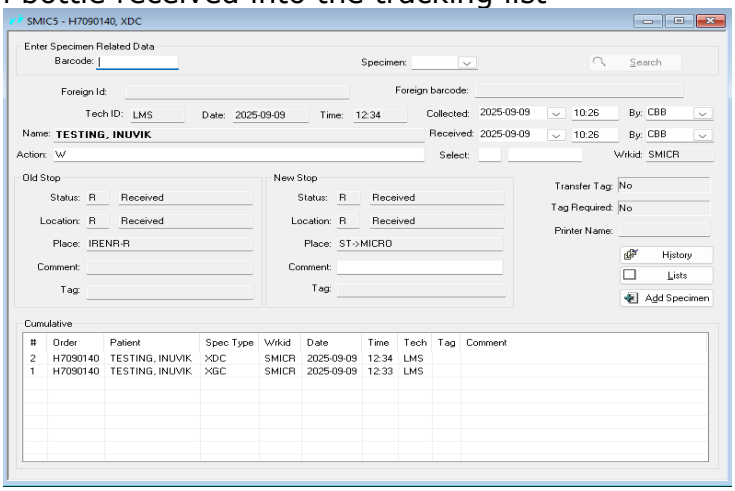
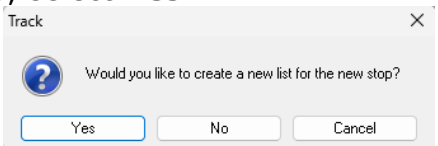
Step	Action
1	Open the order in Results Entry by scanning or entering the accession number of the blood culture order: 
2	In Results Entry: 1. Click on the first bottle in the Test ID column: 
3	In the media resulting area of the order at the bottom part of the screen: 1. Select **Add Media**:  2. In the Select Media box, enter the test ID **24** and select **OK**:  3. The Search Results box appears with 24HRS media ID selected. Select **OK** to add it to the plate log: 

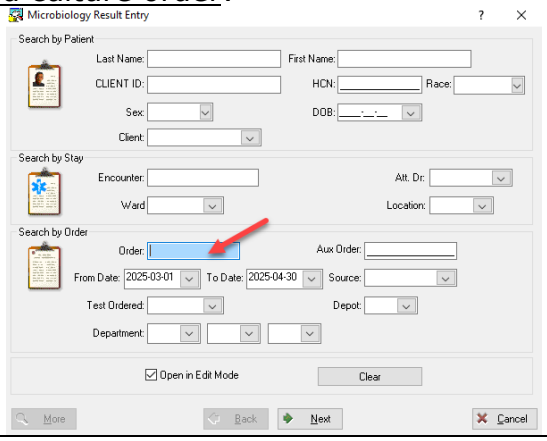
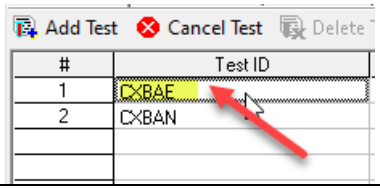
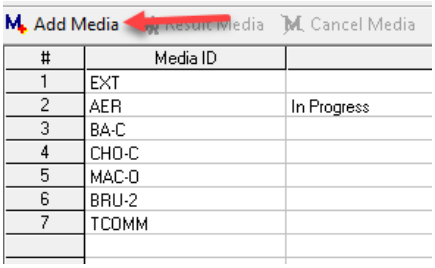
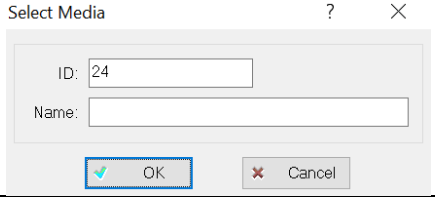
	4. In the Media Comment box, use the keypad to select **Key A** to order the plates to be planted and select **OK**:  5. The Keypad will generate the appropriate plates in the lines below the **24HRS Media ID**:  6. If ANA bottle was also received, repeat the process for the CXBAN order
4	<u>Save changes to the plate log using the Print Subculture Media Label</u>: 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, select **Yes** to save changes
5	<u>Media labels to be printed will be selected</u>: 1. After saving changes the **Select Subculture Media** box generates 2. All required plates are checked off 3. Select **OK**: 

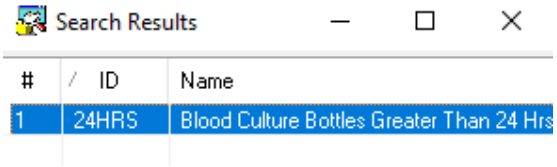
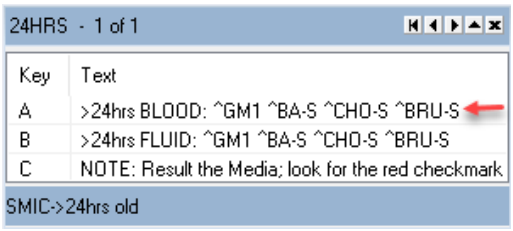
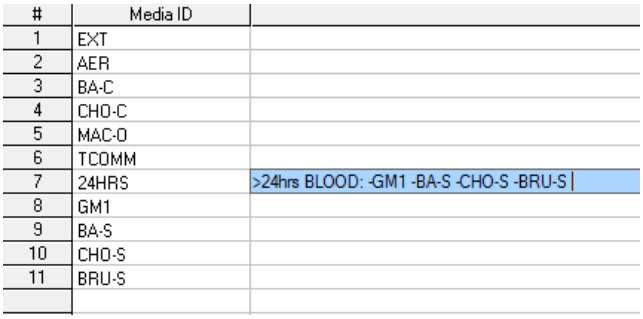
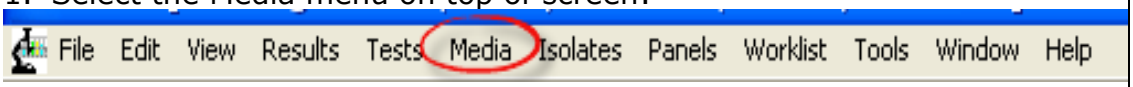
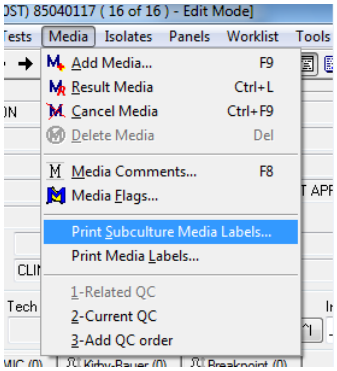
	4. After selecting OK the **Label Properties** box generates 5. Ensure the **Label Layout** matches the example:  **NOTE:** This format only applies to the main microbiology label printer and not the desktop Zebra printers
6	Label the following media/slides: • BA-S: Blood agar • CHO-S: Chocolate agar • 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) • Clean slide with alcohol pad prior to inoculation **NOTE:** Indicate which bottle is >24 hours on ALL plates and slides **NOTE:** Write ">24 HR" on ALL plates and slides
7	Working in the biosafety cabinet subculture the bottle(s): 1. Swab the rubber septum with an alcohol pad. Insert a vent into the bottle 2. Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:  3. Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet 4. Using a sterile loop, streak the plates for isolation 5. Spread the drops out on the FULL slide using the sterile loop: 
8	Load bottles onto the BACTEC FX analyzer as per MIC71000-BACTEC FX Instrument.

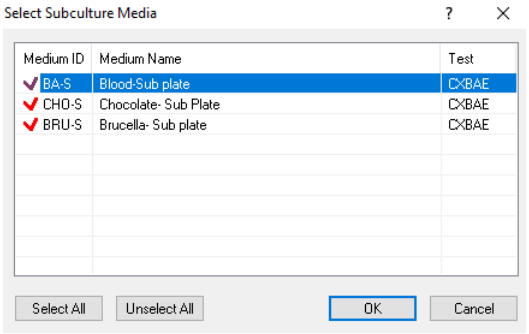
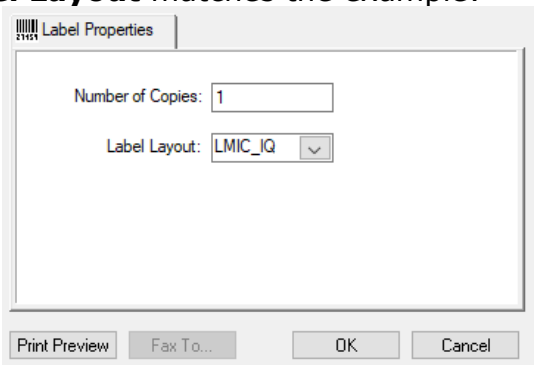
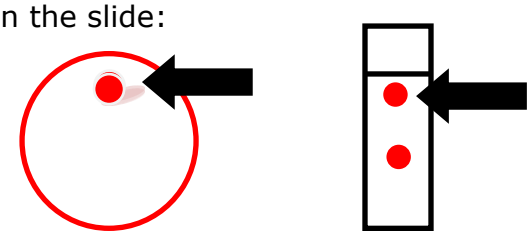
9	Place BA and CHO plates in the lower CO_2 incubator on the "24 HR Blood Cultures" shelf.
10	Place BRU plate in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO$_2$" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
11	Gram stain slide. Refer to MIC20115-Gram Stain.

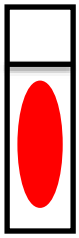
d. Blood Culture received >24 hr from Inuvik Laboratory

Step	Action
1	The Inuvik Laboratory will need to workstation redirect and specimen track the >24 hour bottle to the Stanton Microbiology Laboratory.
2	<u>When the bottles arrive at the Stanton Laboratory, they will need to be tracked to the STH Microbiology Laboratory</u>: 1. In the SoftLab module, open **Specimen Tracking**  2. Select #B R->R->ST-MICRO and select **OK** or double-click on #B  3. Scan each bottle received into the tracking list  4. Once all bottles are scanned, select **Save** on the top tool bar 5. The Track dialogue box will appear asking if you want to create a new list for the new stop, select **Yes**  6. The Print specimen tracking list dialogue box will appear, select **Cancel**

3	<u>After the bottles are tracked to the STH Microbiology Laboratory, they need to be received at the STH micro lab in Order Entry</u>: 1. Enable edit mode by selecting the **Edit Mode** icon on the top toolbar 2. In the Received by line, change the ID to your initials, change the time to the current time and change the date to the current date 3. Select **Save** from the top toolbar to save the new received by information **NOTE:** Ensure that **Specimen Plated** is **NOT** selected
4	After the bottles are received at the STH Microbiology Laboratory, the >24 hour media will need to be added.
5	<u>Open the order in Results Entry by scanning or entering the accession number of the blood culture order</u>: 
6	<u>In Results Entry:</u> 1. Click on the first bottle in the Test ID column: 
7	<u>In the media resulting area of the order at the bottom part of the screen</u>: 1. Select **Add Media**:  2. In the Select Media box, enter the test ID **24** and select **OK**: 

	3. The Search Results box appears with 24HRS media ID selected. Select **OK** to add it to the plate log:  4. In the Media Comment box, use the keypad to select **Key A** to order the plates to be planted and select **OK**:  5. The Keypad will generate the appropriate plates in the lines below the **24HRS Media ID**:  6. If ANA bottle was also received, repeat the process for the CXBAN order
8	<u>Save changes to the plate log using the Print Subculture Media Label</u>: 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, select **Yes** to save changes
9	<u>Media labels to be printed will be selected</u>: 1. After saving changes the **Select Subculture Media** box generates 2. All required plates are checked off

	3. Select **OK**:  4. After selecting OK the **Label Properties** box generates 5. Ensure the **Label Layout** matches the example:  **NOTE:** This format only applies to the main microbiology label printer and not the desktop Zebra printers
10	<u>Label the following media/slides</u>: • 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) • Clean slide with alcohol pad prior to inoculation **NOTE:** Indicate which bottle is >24 hours on ALL plates and slides **NOTE:** Write ">24 HR" on ALL plates and slides
11	<u>Working in the biosafety cabinet subculture the bottle(s)</u>: 1. Swab the rubber septum with an alcohol pad. Insert a vent into the bottle 2. Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:  3. Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet 4. Using a sterile loop, streak the plates for isolation

	5. Spread the drops out on the FULL slide using the sterile loop: 
12	Load bottles onto the BACTEC FX analyzer as per MIC71000-BACTEC FX Instrument.
13	Place BA and CHO plates in the lower CO_2 incubator on the "24 HR Blood Cultures" shelf.
14	Place BRU plate in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO$_2$" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
15	Gram stain slide. Refer to MIC20115-Gram Stain.

3. PROCEDURE INSTRUCTIONS: BLOOD PRODUCT CULTURE

Step	Action
1	• Refer to MIC10300-Blood Product Culture Processing

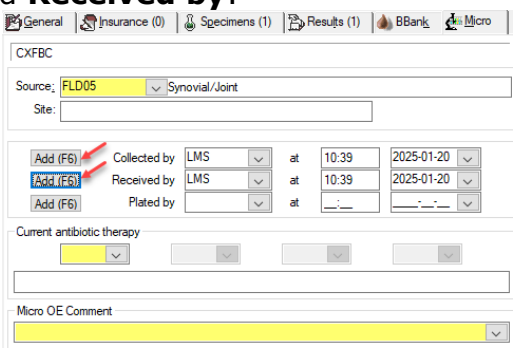
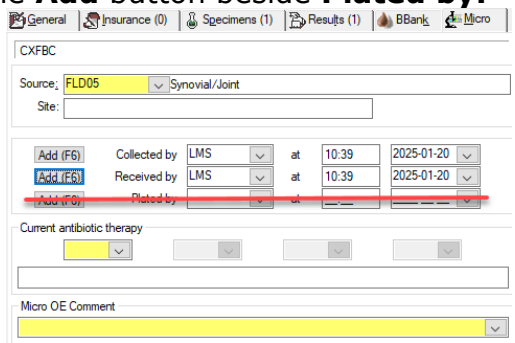
4. PROCEDURE INSTRUCTIONS: BODY FLUID CULTURE

a. Body 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, refer to part 4. b. • If swab is received, add Specimen Quality comment **SWBFL** • Refer prosthetic device specimens for culture to APL • Refer tissue or biopsy specimens for culture to APL
2	Specimen should be stored at room temperature. **NOTE:** If a delay in processing is anticipated, 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 by adding Specimen Quality comment **LEAK** 3. Specimens received in the laboratory in a syringe with the needle still attached will be rejected. In addition, an RL6 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. SCM40110-Waiver of Responsibility form 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: • **>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 • BRU-2: Brucella agar • THIO2: Thioglycollate broth • Label the frosted end of a microscope slide with the accession number, patient's last name and specimen type • Clean slide with alcohol pad prior to inoculation
6	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
7	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
8	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.

9	Place specimen container, supernatant (SUP) tube and sediment (CONC) tube in the O_2 incubator in the "STERILE BODY FLUIDS" bucket.
10	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 fluid is from above the neck, keep THIO and BRU for 10 days
11	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
12	Place BA and CHO plates in the CO_2 incubator on the "NEW WOUND" shelf.
13	Place BRU in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO$_2$" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
14	Gram stain slide. Refer to MIC20115-Gram Stain. **NOTE:** Fluid gram stains should be read within 1 hour of processing during regular Microbiology laboratory hours

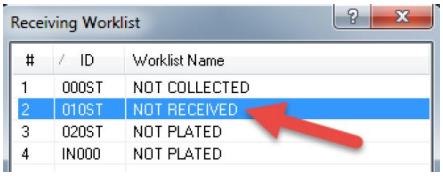
b. Body fluid received in blood culture bottle <24 hours old:

Step	Action
1	• Sterile fluid received in blood culture bottles
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. Broken/cracked bottle **NOTE:** If patient has been treated with antibiotics, fluid specimens in blood culture bottles are considered irretrievable. SCM40110-Waiver of Responsibility form needs to be filled out by the responsible nurse **NOTE:** Except for the above conditions, fluid specimens in blood culture bottles are not rejected regardless of delayed transport, if received frozen or if bottles are expired. Ensure the appropriate specimen quality comments are attached to the specimen in OE and process body fluid in blood culture bottle specimen
4	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 in 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.
5	Receiving can be performed in Order Entry: 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:** 

6

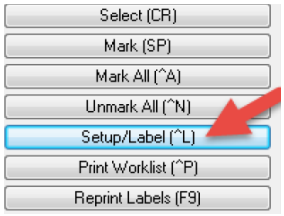
Receiving of multiple bottles can be performed in the Receiving Worklist:

1. Select **Receiving Worklist** icon on the main menu
2. Select **Not Received:**

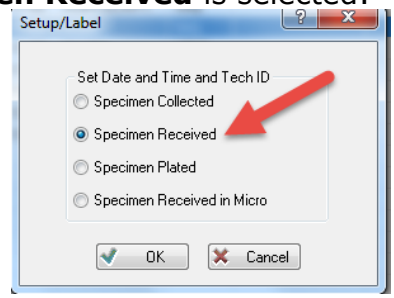


3. Scan the blood culture bottles that you want to receive. Each bottle that has been scanned will have a red check mark beside the order on the left side:

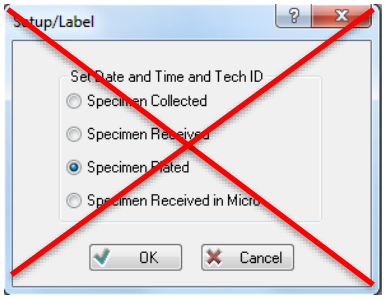
4. Select **Setup/Label** from the menu on the right-hand side:



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

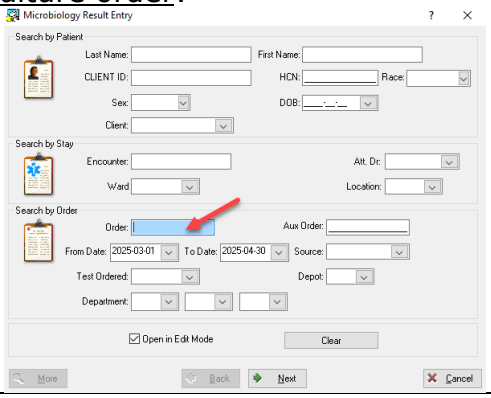
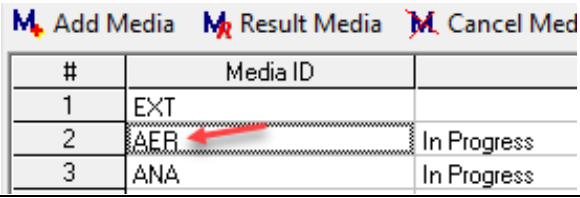
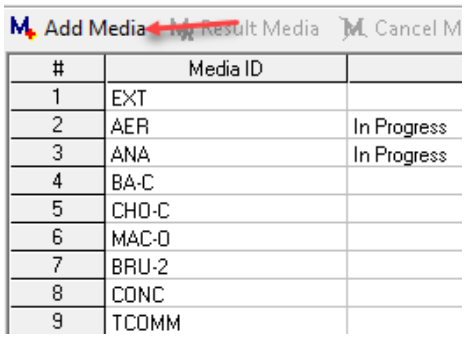
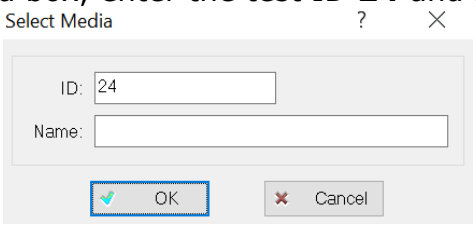
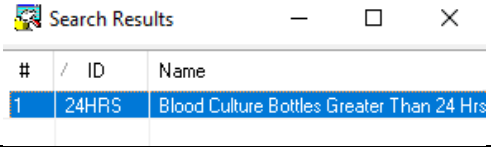


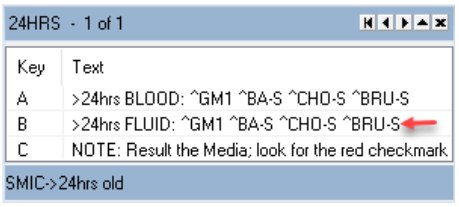
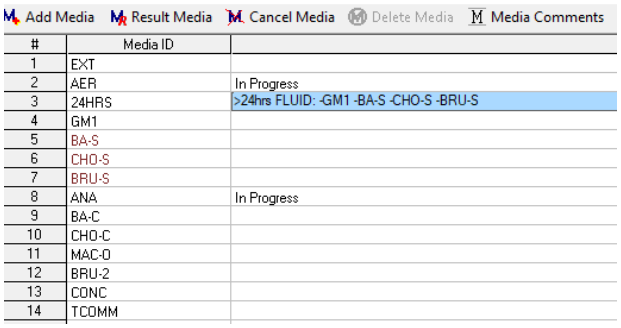
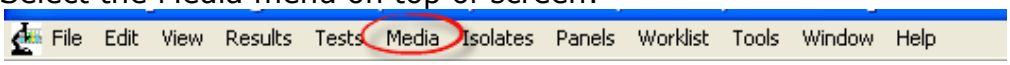
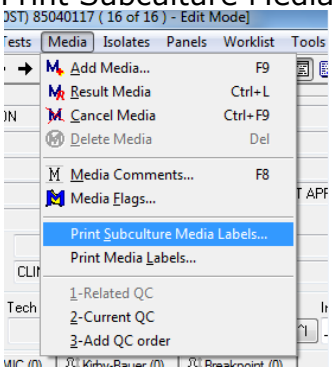
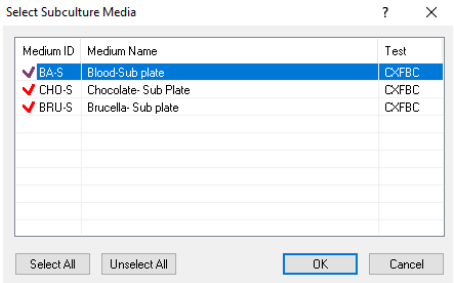
6. Ensure that **Specimen Plated** is **NOT** selected:

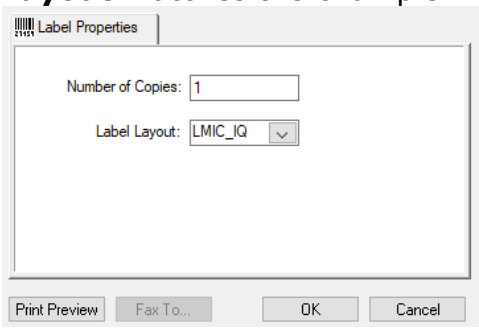
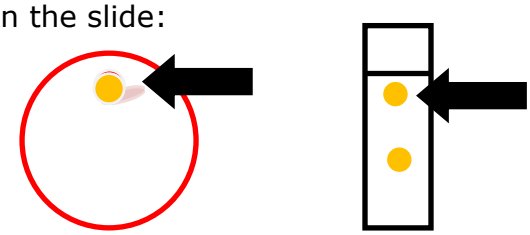
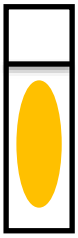


7. Once you have ensured that Specimen Received is selected, select the OK button to receive the specimens
8. Load bottles onto the BACTEC FX analyzer as per MIC71000-BACTEC FX Instrument

c. Body fluid received in blood culture bottle > 24 hours old:

Step	Action
1	<u>Open the order in Results Entry by scanning or entering the accession number of the fluid culture order</u>: 
2	<u>In Results Entry</u>: 1. Click on the first bottle in the Media ID column: 
3	<u>In the media section of the order at the bottom part of the screen</u>: 1. Select **Add Media**:  2. In the Select Media box, enter the test ID **24** and select **OK**:  3. The Search Results box appears with 24HRS media ID selected. Select **OK** to add it to the plate log: 

	4. In the Media Comment box, use the keypad to select **Key B** to order the plates to be planted and select **OK**:  5. The Keypad will generate the appropriate plates in the lines below the **24HRS Media ID**:  6. If ANA bottle was also received, repeat the process for the ANA media
4	<u>Save changes to the plate log using the Print Subculture Media Label</u>: 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, select **Yes** to save changes
5	<u>Media labels to be printed will be selected</u>: 1. After saving changes the **Select Subculture Media** box generates 2. All required plates are checked off 3. Select **OK** 

	4. After selecting OK the **Label Properties** box generates 5. Ensure the **Label Layout** matches the example:  **NOTE:** This format only applies to the main microbiology label printer and not to the desktop Zebra printers
6	<u>Label the following media/slides</u>: • 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) • Clean slide with alcohol pad prior to inoculation **NOTE:** Indicate which bottle is >24 hours on ALL plates and slides **NOTE:** Write "> 24 HR" on ALL plates and slides
7	<u>Working in the biosafety cabinet subculture the bottle(s)</u>: 1. Swab the rubber septum with an alcohol pad. Insert a vent into the bottle 2. Holding the bottle horizontally, place one drop on each plate and two small drops on the slide:  3. Carefully pull the vent out of the bottle and discard it into the sharps container in the biosafety cabinet 4. Using a sterile loop, streak the plates for isolation 5. Spread the drops out on the FULL slide using the sterile loop: 
8	Load bottles onto the BACTEC FX analyzer as per MIC71000-BACTEC FX Instrument.

9	Place BA and CHO plates in the lower CO_2 incubator on the "24 HR Blood Cultures" shelf.
10	Place BRU in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO_2" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
11	Gram stain slide. Refer to MIC20115-Gram Stain.

5. PROCEDURE INSTRUCTIONS: CSF CULTURE

Step	Action
1	• Central nervous system shunt fluid • CSF from lumbar puncture
2	Specimen should be stored at room temperature. **NOTE:** If a delay in processing is anticipated, 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 by adding Specimen Quality comment **LEAK** 3. Improperly collected, labeled, transported or handled specimens should be processed. SCM40110-Waiver of Responsibility form 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
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 pad prior to inoculation **NOTE:** If specimen is from a shunt, THIO needs to be added
6	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place specimen collection tube and supernatant tube (if applicable) in the O_2 incubator in "STERILE BODY FLUIDS" bucket.
9	If applicable, label THIO with Day 2 date, Day 5 date, Day 10 date and the Day 14 date. Place THIO broth in "THIO" rack in O_2 incubator in "Day 2" row.
10	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
11	Place BA and CHO plates in the CO_2 incubator on the "NEW WOUND" shelf.
12	Gram stain slide. Refer to MIC20115-Gram Stain. **NOTE:** CSF gram stains should be read within 1 hour of processing during regular Microbiology hours

6. PROCEDURE INSTRUCTIONS: EAR CULTURE

Step	Action
1	• External auditory canal (outer ear) • Otitis media discharge swabbed from external auditory canal **NOTE:** Tympanocentesis fluid should be ordered as a body fluid culture
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
4	Label the following media/slides: • BA-C: Blood agar • CHO-C: Chocolate agar • CNA-C: Colistin-nalidixic acid 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 MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
9	Place BA, CHO and CNA plates in the CO_2 incubator on the "NEW WOUND" shelf.
10	Gram stain slide. Refer to MIC20115-Gram Stain.

7. PROCEDURE INSTRUCTIONS: EYE CULTURE

a. Superficial Eye

Step	Action
1	• Conjunctiva • Superficial corneal 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
4	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
5	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place BA and CHO plates in the CO_2 incubator on the "NEW WOUND" shelf.
9	Gram stain slide. Refer to MIC20115-Gram Stain.

b. Deep Eye

Step	Action
1	• Corneal scrapings **NOTE:** Aqueous/vitreous fluid should be ordered as a body fluid culture
2	Specimen should be stored at room temperature.
3	Criteria for rejection: 1. Unlabeled/mislabeled swabs 2. Specimen container label does not match patient identification on requisition 3. Improperly collected, labeled, transported or handled specimens should be processed. SCM40110-Waiver of Responsibility form needs to be filled out by the responsible nurse
4	Label the following media/slides: • BA-C: Blood agar • CHO-C: Chocolate agar • THIO2: Thioglycollate broth • Label the frosted end of a microscope slide with the accession number, patient's last name and specimen type • Clean slide with alcohol pad prior to inoculation
5	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-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.
9	Place BA and CHO plates in the CO_2 incubator on the "NEW WOUND" shelf.
10	Gram stain slide. Refer to MIC20115-Gram Stain. **NOTE:** Deep eye stains should be read within 1 hour of processing during regular Microbiology hours
11	If a physician requests media for a corneal scraping, remove the necessary supplies from the Corneal Scraping Kit on the specimen-receiving cart and place them in a biohazard bag. Add two clean glass slides to the plastic slide box and include it in the bag as well. When the inoculated media is returned to the microbiology laboratory, ensure it is labeled with the patient's name and, ideally, a second identifier. If any media is improperly labeled, the SCM40110 – Waiver of Responsibility form must be completed by the responsible nurse. Incubate the inoculated media as described above. **NOTE:** If no inoculated glass microscope slides are returned, result the STGM1 order using the GRAM Test Comment: "No smear received, test not performed" and finalize the order

8. PROCEDURE INSTRUCTIONS: GBS SCREEN

Step	Action
1	• Vaginal-Rectal • 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. Duplicate specimens obtained with same collection method from same collection location within 24 hours
4	Label the following media/slides: • 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 technologist performing daily shutdown duties
7	After the LIM broth incubates for 18-24 hours: • 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 • Also label the GBS-O plate with R: Date +2 date • 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 StrepB*Select* agar ➢ Streak for isolated growth using a disposable inoculation needle ➢ Streak out to cover the whole plate
8	Place GBS plate on the "GBS Screens" shelf in the O_2 incubator.

9. 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/slides: • THIO2: Thioglycollate broth • BRU-2: Brucella agar **NOTE:** 2 THIO broths will be needed for IUD processing
4	Add the extra thioglycollate broth tube to the specimen container containing the IUD and vortex for 30 seconds.
5	Using a sterile pipette, transfer the THIO broth from the sterile container into a red top tube labeled with CONC label and centrifuge at 3500 rpm for 10 minutes.
6	After centrifugation is complete, remove supernatant with sterile pipette and place into red top tube labeled with SUP label.
7	Using the sediment, inoculate the media: • 1 drop on BRU • 2-5 drops in labelled THIO broth
8	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
9	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.
10	Place BRU in anaerobic jar or tray with anaerobic pouch and indicator as soon as practical after inoculation. Label jar or tray with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling.
11	Place supernatant (SUP) tube and sediment (CONC) tubes in the MAC in the O_2 incubator
12	Gram stain is not performed. No slide is required.

10. PROCEDURE INSTRUCTIONS: MRSA SCREEN

Step	Action
1	• Bilateral nasal swab • Bilateral groin swab • 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	Label the following media/slides: • MRS-O: MRSA*Select* II agar
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label the MRSA plate with R: Date +1 date
8	Place MRS plate in the O_2 incubator on the "NEW URINE " shelf.

11. PROCEDURE INSTRUCTIONS: MRO SCREEN

Step	Action
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	Label the following media/slides: • MRS-O: MRSA*Select* II agar • VRE-O: VRE*Select* agar
5	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	• Label the MRSA plate with R: Date +1 date • Label the VRE plate with R: Date +2 date
8	Place MRS plates in the O_2 incubator on the "NEW URINE" shelf. Place VRE plates on the "VRE SCREENS" shelf in the O_2 incubator.

12. PROCEDURE INSTRUCTIONS: NASAL 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
4	Label the following media/slides: • BA-O: Blood agar • CNA-O: Colistin-nalidixic acid agar
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label the BA and CNA plates with: R: Date +2 date.
8	Place BA and CNA plates on the "NOSE CULT" shelf in the O_2 incubator.
9	Gram stain is not performed. No slide is required.

13. PROCEDURE INSTRUCTIONS: *NEISSERIA GONORRHOEAE* CULTURE

Step	Action
1	• Urethra • 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
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 **NOTE:** Slides are only made on urethra specimens
5	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	If applicable, make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place CHO and TM plates in the CO_2 incubator on the "NEW URINE" shelf.
9	If applicable, gram stain slide. Refer to MIC20115-Gram Stain.

15. 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
4	Label the following media/slides: • YST-O: Candi*Select* agar
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label YST plate with R: Date +2 date.
8	Place YST plate on the "YST SCREENS" shelf in the O_2 incubator.
9	Gram stain is not performed. No slide is required.

16. PROCEDURE INSTRUCTIONS: RESPIRATORY CULTURE

Step	Action
1	• Sputum • Endotracheal aspirate • Auger suction • Bronchial aspirates (washings) • Bronchoalveolar lavage (BAL)
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. Swabs of sputa 4. Duplicate specimens obtained with the same collection method within 24 hours 5. Leaking specimens 6. Improperly collected, labeled, transported or handled bronchial aspirate (wash specimens), BAL specimens, lung aspirates and lung biopsy specimens should be processed. SCM40110-Waiver of Responsibility form needs to be filled out by the responsible nurse.
4	Label the following media/slides: • BA-C: Blood agar • CHO-C: Chocolate agar • CNA-C: Colistin-nalidixic acid 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 MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
9	Place BA, CHO and CNA plates in the CO_2 incubator on the "NEW WOUND shelf.
10	Gram stain slide. Refer to MIC20115-Gram Stain.

17. 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	Label the following media/slides: • BA-2: Blood agar
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label BA plate with R: Date +2 date.
8	Place BA plate in "For throat jar" rack in the CO_2 incubator.
9	Gram stain is not performed. No slide is required.

18. PROCEDURE INSTRUCTIONS: TIP CULTURE

Step	Action
1	• Intravascular catheters including: central, CVC, peripheral, arterial, jugular, femoral, subclavian, umbilical and hemodialysis
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 4. Chest tube tips and abdominal drain tips 5. Catheter tips should not be placed in saline or transport medium
4	Label the following media/slides: • BA-C: Blood agar • MAC-O: MacConkey agar
5	Using a sterile needle, roll the segment back and forth 4 times across the surface of the Blood agar plate followed by the MacConkey plate. **NOTE:** 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 the "NEW WOUND" shelf.
7	Place BA plate in the CO_2 incubator on the "NEW WOUND" shelf.
8	Gram stain is not performed. No slide is required.

19. PROCEDURE INSTRUCTIONS: Toxigenic *C. difficile*

Step	Action
1	• Refer to MIC72400-Xpert *C. difficile*

20. PROCEDURE INSTRUCTIONS: *Trichomonas* Rapid Test

Step	Action
1	• Refer to MIC10350-OSOM *Trichomonas* Rapid Test

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 >24 hours after collection 5. 24 hour urine collections 6. Foley catheter tips 7. Specimens in leaking container
4	Label the following media/slides: • UR1-O: Uri*Select* 4 agar for non-sterile urine specimens • UR2-O: Uri*Select* 4 agar for sterile urine specimens **NOTE:** Highlight urine type on plate if UR2-O
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Place URI plate in the O_2 incubator on the "NEW URINE" shelf.

22. PROCEDURE INSTRUCTIONS: VRE SCREEN

Step	Action
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. Nasal and axilla swabs should not be processed for VRE
4	Label the following media/slides: • VRE-O: VRE*Select* agar
5	Inoculate plates with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label the VRE plate with: R: Date +2 date.
8	Place VRE plate on the "VRE SCREENS" shelf in the O_2 incubator.

23. PROCEDURE INSTRUCTIONS: WET PREP SCREEN

Step	Action
NOTE: Wet prep is only performed in absence of *Trichomonas* Rapid Test	
1	• Cervix • Urethra (male) • Vagina
2	Specimen should be stored at room temperature.
3	Criteria for rejection: 1. Specimen is >72 hours old 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
4	Label the following media/slides: • 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 at least 15 minutes.

24. PROCEDURE INSTRUCTIONS: WOUND CULTURE

a. Superficial Wound

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	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. Specimens for culture submitted in container with formalin 4. Submission of specimens to determine *if* an infection is present should be discouraged
4	Label the following media/slides: • BA-C: Blood agar • CNA-C: Colistin-nalidixic acid 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 MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
9	Place BA and CNA plates in the CO_2 incubator on the "NEW WOUND" shelf.
10	Gram stain slide. Refer to MIC20115-Gram Stain.

b. Deep Wound

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. Specimens for culture submitted in container with formalin
4	Label the following media/slides: • BA-C: Blood agar • CHO-C: Chocolate agar • CNA-C: Colistin-nalidixic acid agar • MAC-O: MacConkey agar • BRU-2: Brucella agar • KV-2: Anaerobic KV 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 MIC10000-Microbiology Specimen Handling.
6	Make Gram stain smear. Refer to MIC10000-Microbiology Specimen Handling.
7	Streak plates for isolation. Refer to MIC10000-Microbiology Specimen Handling.
8	Place MAC plate in the O_2 incubator on the "NEW WOUND" shelf.
9	Place BA, CHO and CNA plates in the CO_2 incubator on the "NEW WOUND" shelf.
10	Place BRU and KV plates in an anaerobic tray or jar with anaerobic pouch and indicator as soon as practical after inoculation. Label tray or jar with date of 48 hour read and place in the O_2 incubator on the "WOUND ANO$_2$" shelf. **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation. Refer to MIC10000-Microbiology Specimen Handling
11	Gram stain slide. Refer to MIC20115-Gram Stain.

25. PROCEDURE INSTRUCTIONS: YEAST CULTURE

Step	Action
1	• Anal • Cervix • Penis • Vagina **NOTE:** Refer to MIC10110-Vaginal Swab Processing Job Aid for yeast culture 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. Vaginal swab without appropriate clinical history
4	Label the following media/slides: • YST-O: Candi*Select* agar
5	Inoculate plate with specimen. Refer to MIC10000-Microbiology Specimen Handling.
6	Streak plate for isolation. Refer to MIC10000-Microbiology Specimen Handling.
7	Label YST plate with R: Date +2 date.
8	Place YST plate on the "YST SCREENS" shelf in the O_2 incubator.
9	Gram stain is not performed. No slide is required.

CROSS-REFERENCES:

- MIC10000-Microbiology Specimen Handling
- MIC10110-Vaginal Swab Processing Job Aid
- MIC10300-Blood Product Culture Processing
- MIC10350-OSOM *Trichomonas* Rapid Test
- MIC20115-Gram Stain
- MIC60010-Microbiology Quality Control procedure
- MIC60040-Culture Media Quality Control procedure
- MIC71000-BACTEC FX Instrument
- SCM40100-Specimen Acceptance and Rejection Policy
- SCM40110-Waiver of Responsibility Form

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4thed.) Washington, D.C.: ASM Press
2. 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*. Washington, D.C: ASM Press

APPROVAL:

March 15, 2024
Date

Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	11 Aug 2016	Initial Release	L. Steven
2.0	10 Jun 2019	Update to reflect new urine chromogenic agar	L. Steven
3.0	27 Feb 2020	Procedure reviewed	L. Steven
4.0	30 Jan 2022	Procedure reviewed and added to NTHSSA policy template	L. Steven
5.0	14 Feb 2024	Procedure reviewed	L. Steven