PURPOSE:

A guide to the processing of specimens submitted for bacterial culture. This procedure will instruct on the following samples:

1. Blood Cultures
   a. Positive Blood Cultures
   b. Blood Cultures received >24 hour
2. CSF
3. Sterile fluids (not CSF/Blood Cultures)
4. Urines
5. Stools
6. Superficial Wounds/Ears
7. Deep Wounds/Miscellaneous aspirates
8. Eyes
   a. Superficial
   b. Deep
9. Throats
10. Sputum/ETT/Bronchial Washes
11. Genital Cultures
12. MRO swabs
13. Group B Screen
14. IUD
15. Catheter tips
16. Oral Cultures

REAGENTS and/or MEDIA:

Blood agar (BA)
MacConkey agar (MAC)
Chocolate agar (CHOC)
Brucella agar (BAA)
Laked blood and KV (KVL)
Colistin-nalidixic agar (CAN)
Campy agar (Campy)
Sorbitol MacConkey agar (SMAC)
Cefsulodin, Irgasan, Novobiocin agar (CIN)
Hektoen agar (HEK)
Selenite broth (SEL)
Thayer Martin agar (TM)
LIM GrpB broth
Thio broth
Sabouraud agar (SAB)
Cooked meat broth
Denium Blue agar (MRSA)
Colorex VRE

SUPPLIES:

Spreading loops/ straight wires
Glass slides
Calibrated 1µL blue loops
Sterile pipettes
Cotton tipped swabs
Anaerobic/Microaerophilic Jars
Anaerobic indicator
Anaerogen pack
Campygen pack

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 MIC60100 Non-Exempt Media Quality Control procedure

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:

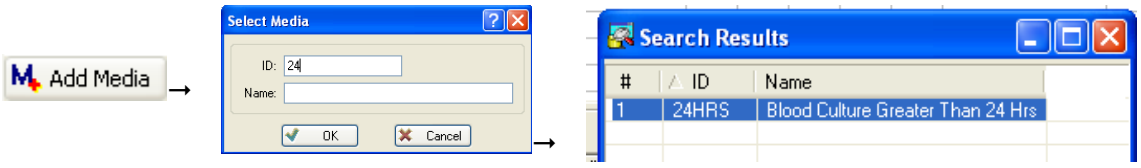
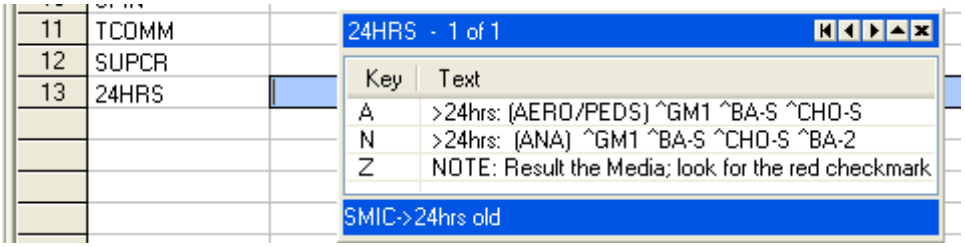
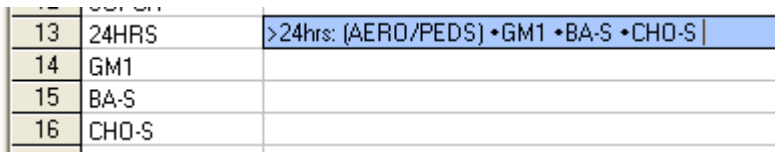
- False-positive cultures result from specimen mix-up and from contamination of media used for culture
- False-negative results are due to improper collection, delays in culture inoculation, inappropriate medium usage and inappropriate incubation conditions

1. PROCEDURE INSTRUCTIONS: BLOOD CULTURES

a. Positive Blood Cultures in Bactec FX

Step	Action
1	Remove positive blood culture bottle(s) from the Bactec FX
2	**Generate plate labels through the LIS system**: • In Order Entry: Enter Accession # → Micro Tab → Hit F6 in the "Plated By" field → SAVE
3	**Label media**: • Aerobic/Pediatric Bottle: Blood agar, Chocolate agar and MacConkey agar • Anaerobic Bottle: Blood agar, Chocolate agar, MacConkey agar and Blood agar plate incubated anaerobically • 1 glass slides labeled with the accession number, patient's last name, bottle type (AE/AN/PEDS), specimen type (BLD) **NOTE:** Please indicate which bottle is positive on ALL plates and slides i.e. AE for Aerobic bottle, AN for Anaerobic bottle, PEDS for Pediatric bottle
4	**Working in the biosafety cabinet subculture the vial(s):** • Swab the rubber septum with an alcohol prep pad • Insert a vent into the vial • Holding the vial horizontally, place one drop on each plate and one small drop on the slide • Carefully pull the vent out of the vial and discard it into the sharps container in the biosafety cabinet • Using a loop, streak the plates for isolation and then spread the drop out on the FULL slide
5	**Incubate the inoculated plates/media as follows:** • BA-C and CHO-C: CO_2 incubator in white tray labeled "Blood Culture" • MAC-O: O_2 incubator at 35ºC in white tray labeled "MAC – Pos Blood Culture" • BA-2 : Anaerobic jar, O_2 incubator • Blood Culture Bottle: place in top shelf of O_2 incubator
6	Allow the slides to dry on slide warmer
7	Gram stain as per Gram-stain procedure MIC20115

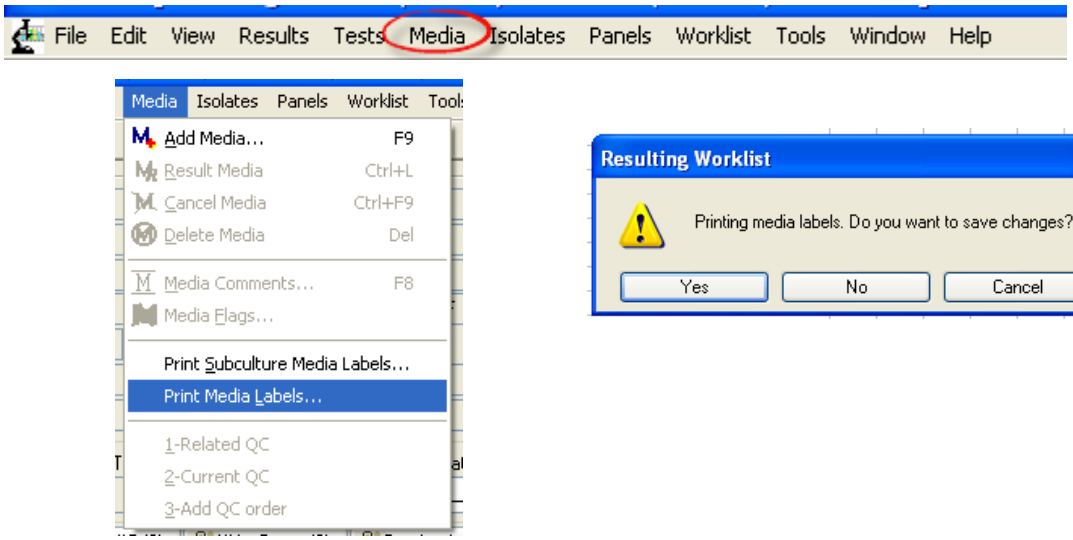
b. Blood Cultures received >24 hour

Step	Action
1	**Add the Plate Log code: "24"** • Click on "Add Media" → in the 'ID" field type in "24" → Search Results screen pops up with the name of the 24HRS media ID → click OK to add it to the plate log (see below). 
2	**Add >24 plates to the plate log:** • In the Media Comment line, use the keypad to select the appropriate plates depending on bottle  <u>Aerobic/Pediatric bottle:</u> • GM1 • BA-C and CHO-C (blood and chocolate plates into CO2) <u>Anaerobic bottle:</u> • GM1 (>24 hr gram stain) • BA-C and CHO-C (blood and chocolate plates into CO2) • BA-2 (blood plate into Anaerobic Tray) • Keypad will generate appropriate plates in the lines below the 24 HRS media code (see examples below): Example of a >24HR received aerobic or pediatric bottle: 

3

Save changes to the plate log using the Print Media Label follow-thru:

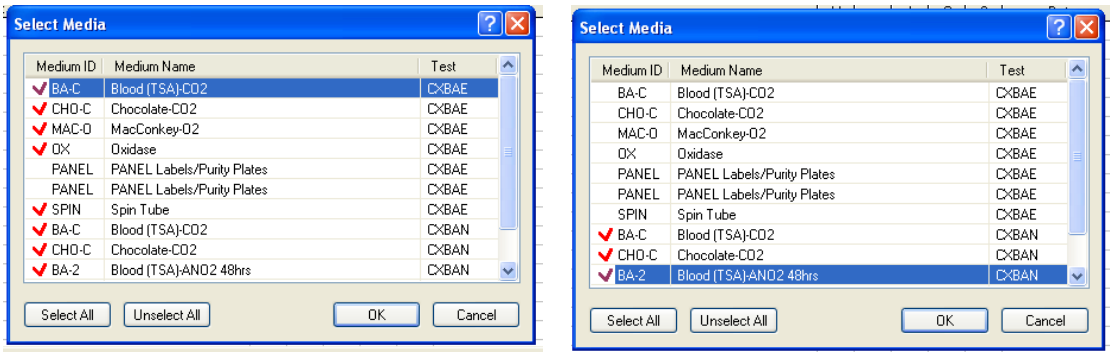
- Click on the Media menu on top of screen → Scroll down and click on Print Media Labels → pop-up box asks to save changes → click "Yes" to save your >24 hour media additions to the plate log (see below).

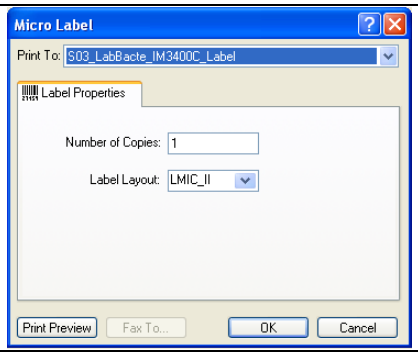


4

Select media plates to be printed:

- After saving changes to plate log a Select Media box generates → Click "Unselect All" → Select the appropriate plates for the blood culture vial → click 'OK"



5	After selecting the appropriate label and clicking OK → Micro Label box generates → Ensure the format matches with the format in the example to the right: 
6	**Label media**: • Aerobic/Pediatric Bottle: Blood agar, Chocolate agar and MacConkey agar • Anaerobic Bottle: Blood agar, Chocolate agar, MacConkey agar and Blood agar plate incubated anaerobically. • 1 glass slides labeled with the accession number, patient's last name, bottle type (AE/AN/PEDS), specimen type (BLD) **NOTE:** Please indicate which bottle is positive on ALL plates and slides i.e. AE for Aerobic bottle, AN for Anaerobic bottle, PEDS for Pediatric bottle.
7	**Working in the biosafety cabinet subculture the vial(s):** • Swab the rubber septum with an alcohol prep pad. • Insert a vent into the vial. • Holding the vial horizontally, place one drop on each plate and one small drop on the slide. • Carefully pull the vent out of the vial and discard it into the sharps container in the biosafety cabinet. • Using a loop, streak the plates for isolation and then spread the drop out on the FULL slide.
8	**Incubate the inoculated plates/media as follows:** • BA-C and CHO-C: CO_2 incubator in white tray labeled "Blood Culture" • MAC-O: O_2 incubator at 35ºC in white tray labeled "MAC – Pos Blood Culture" • BA-2 : Anaerobic jar, O_2 incubator • Blood Culture Bottle: Place into Bactec FX
9	Allow the slides to air-dry under the biosafety cabinet.
10	Gram stain as per Gram-stain procedure MIC20115

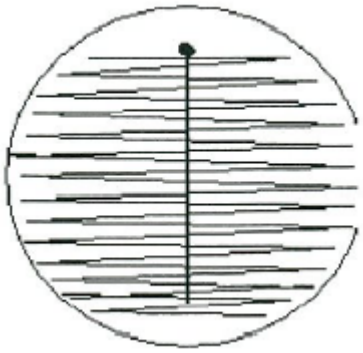
2. PROCEDURE INSTRUCTIONS: CSF

Step	Action
1	Volume received: (Tube 2 is the usual tube for Microbiology) • **>1mL**: Centrifuge at 3500 rpm for 10 minutes (Program 2). Decant supernatant into the AccelTB waste container • **<=1mL**: Inoculate plates using a sterile pipette **NOTE**: If sample is NOT centrifuged → add Specimen Quality comment: "Sample not concentrated"
2	**Label the following media/slides:** • BA-C label: Blood Agar Plate • CHO-C label: Chocolate Plate • MAC-O label: MacConkey Plate • 2 labeled ringed cytology slides with the accession number, patient's last name, and specimen type
3	**Using a STERILE pipette, dispense the fluid sediment as follows:** • 1 drop per plate • 1 drop per slide in the circle area of the slide. Allow slides to dry on the slide warmer
4	Streak all plates for isolation
5	Place the remaining sample sediment in the O_2 incubator
6	**Incubate the inoculated plates/media as follows:** • BA-C, CHO-C: CO_2 incubator, in tray for CSF • MAC-O: O_2 incubator, in tray for CSF
7	Perform Acridine Orange stain (MIC20100) on one smear Perform Gram Stain (MIC20115) on the remaining smear
8	**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 a -70ºC

3. PROCEDURE INSTRUCTIONS: STERILE FLUIDS (NOT CSF/BLOOD)

Step	Action
1	Volume received: (Tube 2 is the usual tube for Microbiology) • **>1mL**: Centrifuge at 3500 rpm for 10 minutes (Program 2). Decant supernatant into the AccelTB waste container • **<=1mL**: Inoculate plates using a sterile pipette **NOTE**: If sample is NOT centrifuged → add Specimen Quality comment: "Sample not concentrated"
2	**Label the following media/slides:** • BA-C label: Blood Agar Plate • BRU-2 label: Brucella Agar • LKV-2 label: LKV Agar • CHO-C label: Chocolate Plate • MAC-O label: MacConkey Plate • THIO2 label: Thioglycollate broth ➢ Indicate on the label "2D: {Date 2 days from planting} and "5D: {Date 5 days from planting}" • 2 labeled ringed cytology slides with the Accession Number, Patient's last name, and specimen type
3	**Using a STERILE pipette, dispense the fluid sediment as follows:** • 1 drop per plate • 1 drop per slide in the circle area of the slide. Allow slides to dry on the slide warmer • 2 – 5 drops in Thioglycollate broth. ➢ On the THIO label indicate – 2D: (Date 2 days later), 5D: (Date 5 days later)
4	Streak all plates for isolation
5	Place the remaining sample sediment in the O_2 incubator
6	**Incubate the inoculated plates/media as follows:** • BA-C, CHO-C, CAN-C: CO_2 incubator • MAC-O: O_2 incubator • BRU-2, LKV-2, CAN-2: Anaerobic jar, O_2 incubator, 48 hours • THIO-2: O2 incubator
7	Perform Acridine Orange stain (MIC20100) on one smear Perform Gram Stain (MIC20115) on the remaining smear

4. PROCEDURE INSTRUCTIONS: URINE CULTURES

Step	Action
1	**Label the following media:** Blood Agar Plate: BA-O or *BA-C label Chocolate Plate: *CHO-C (if specified) MacConkey Plate: MAC-O
2	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
3	Inoculate with the loop down the centre of the plate and then cross-streak at a 90 degree angle to the inoculum  The same loop can be used for each plate per patient sample. The loop must be re-dipped for every plate and least selective plates streaked first
4	**Incubate the inoculated plates as follows:** • BA-O, MAC-O: O_2 incubator • BA-C, CHO-C: CO_2 incubator **NOTE**: If incubating after 6pm, plates must be placed in a rack that specifies that they were incubated after 6pm to ensure adequate incubation time is reached

5. PROCEDURE INSTRUCTIONS: STOOL CULTURES

Step	Action
1	**Label the following media:** • Blood agar, MacConkey agar, Sorbitol MacConkey agar, Hektoen agar x2, Campy agar, Yersinia agar and Selenite broth
2	**Use a cotton-tipped applicator to mix the stool sample and inoculate each plate:** • Lightly inoculate BA, MAC, SMAC • Use heavier inoculums for the HEK-O, CAM-M and CIN-R as they are more selective
3	Place the heavily saturated applicator in the pre-labeled selenite broth and break off the stick to fit. Recap the tube loosely.
4	Streak for isolation
5	**Incubate the inoculated plates/ media as follows:** • BA-O, MAC-O, HEK-O: O_2 incubator • CAM-M: Microaerophilic jar, 42ºC incubator → 72hrs • CIN-R: Room Temperature, indicate on label the date to read R: 48hrs later • SEL-O**:** O_2 incubator **NOTE:** If incubating after 6pm, plates must be placed in a rack that specifies that they were incubated after 6pm to ensure adequate incubation time is reached

6. PROCEDURE INSTRUCTIONS SUPERFICIAL WOUNDS/EARS

Step	Action
1	**Label the following media:** • BA-C, CHO-C, CAN-C: CO_2 incubator • MAC-O: O_2 incubator • 1 glass slides labeled with the accession number, patient's last name and specimen type
2	Remove the swab from the transport container and swab a quarter-sized inoculum to the left hand side of the plate. Rotate the swab to ensure all areas of the swab are planted on the plate. Inoculate all plates in this manner.
3	Inoculate the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on the slide warmer
4	Streak all plates for isolation plates for isolation.
5	**Incubate the inoculated plates as follows:** • BA-C, CHO-C: CO_2 incubator • MAC-O: O_2 incubator
6	Gram stain as per Gram-stain procedure MIC20115

7. PROCEDURE INSTRUCTIONS: DEEP WOUNDS/ASPIRATES

Step	Action
1	**Label the following media/slides:** • BA-C label: Blood agar • CNA-C label AND CNA-2 label: CNA agar • BRU-2 label: Brucella agar • LKV-2 label: LKV agar • CHO-C label: Chocolate agar • MAC-O label: MacConkey agar • Thioglycollate broth: THIO-2 – add "2D: {Date 2 days from planting}" and "5D: {Date 5 days from planting}" • Label the frosted end of a glass slide with the Accession Number, Patient's last name, and specimen type **NOTE:** Plates are determined based on source. Not all plates will be set up for deep wounds/aspirates
2	Remove the swab from the transport container and swab a quarter-sized inoculum to the left hand side of the plate. Rotate the swab to ensure all areas of the swab are planted on the plate. Inoculate all plates in this manner.
3	Inoculate the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on the slide warmer
4	Inoculate a Thioglycollate broth by breaking the swab into the broth or dispensing 2-5 drops of fluid into the broth • On the THIO label indicate – 2D: (Date 2 days later), 5D: (Date 5 days later)
5	Streak plates for isolation
6	**Incubate the inoculated plates/media as follows:** • BA-C, CHO-C, CNA-C: CO_2 incubator • MAC-O: O_2 incubator • BRU-2, LKV-2, CNA-2: Anaerobic jar, O_2 incubator, 48hours • THIO-2: O_2 incubator
7	Gram stain as per Gram-stain procedure MIC20115

8. PROCEDURE INSTRUCTIONS: EYES

a. Superficial eye: conjunctiva, superficial corneal specimens

Step	Action
1	**Label the following media:** • BA-O: Blood agar • CHO-C: Chocolate agar • Label the frosted end of a glass slide with the Accession Number, Patient's last name, and specimen type
2	Remove the swab from the transport container and swab a quarter-sized inoculum to the left hand side of the plate. Rotate the swab to ensure all areas of the swab are planted on the plate. Inoculate all plates in this manner.
3	Inoculate the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on the slide warmer
4	Streak plates for isolation
5	Incubate the inoculated plates as follows: • BA-C, CHO-C: CO_2 incubator
6	Gram stain 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 label: Blood agar • Chocolate Plate: CHO-C label • Brucella Agar: BRU-2 label • LKV Agar: KV-2 label • MacConkey Plate: MAC-O label • Thioglycollate broth: THIO-2 – add "2D: {Date 2 days from planting}" and "5D: {Date 5 days from planting}" • Label the frosted end of a glass slide with the Accession Number, Patient's last name, and specimen type
2	Remove the swab from the transport container and swab a quarter-sized inoculum to the left hand side of the plate. Rotate the swab to ensure all areas of the swab are planted on the plate. Inoculate all plates in this manner.
3	Inoculate the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on the slide warmer
4	Inoculate a Thioglycollate broth by breaking the swab into the broth or dispensing 2-5 drops of fluid into the broth • On the THIO label indicate – 2D: (Date 2 days later), 5D: (Date 5 days later)
5	Streak plates for isolation
6	**Incubate the inoculated plates/media as follows:** • BA-C, CHO-C: CO_2 incubator • MAC-O: O_2 incubator • BRU-2, KV-2: Anaerobic jar, O_2 incubator, 48hours • THIO-2: O_2 incubator
7	Gram stain as per Gram-stain procedure MIC20115

9. PROCEDURE INSTRUCTIONS: THROATS

Step	Action
1	**Label the following media:** • BA-1: Blood agar
2	Remove the swab from the transport container and swab a quarter-sized inoculum to the left hand side of the plate. Rotate the swab to ensure all areas of the swab are planted on the plate. Inoculate all plates in this manner.
3	Streak for isolation
4	**Incubate the inoculated plate as follows:** • BA-1: Anaerobic jar, O_2 incubator

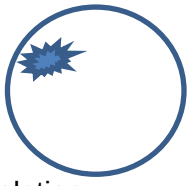
10. PROCEDURE INSTRUCTIONS: SPUTUMS/ETT/BRONCHIAL WASHES

Step	Action
1	**Label the following media/slides:** • BA-C label: Blood agar • CHO-C label: Chocolate agar • MAC-O label: MacConkey agar • Label the frosted end of the slide with Accession Number, Patient's last name, and specimen type (SP)
2	Use a swab to select the most mucoid or muco-purulent parts of the specimen
3	Inoculate each plate and the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on slide warmer
4	Streak for isolation. Add a Staph streak to the Blood Plate.
5	**Incubate the inoculated plates as follows:** • BA-C, CHO-C: CO_2 Incubator • MAC-O: O_2 ambient incubator
6	Gram stain as per Gram-stain procedure MIC20115

11. PROCEDURE INSTRUCTIONS: GENITAL CULTURES

Step	Action
1	**Label the following media/slide:** • BA-C: Blood agar • CHO-C: Chocolate agar • TM-C: Thayer Martin agar • MAC-O: MacConkey agar • Label the frosted end of the slide with the Accession Number, Patient's last name, and specimen type **Slides are made on the following samples:** • Genital Culture (CXGEN) • Gonorrhea Culture (CXGON): site Urethra ➢ **Slides are not made on cervix samples.**
2	Inoculate each plate and the slide by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. Allow slide to dry on slide warmer if one was required
3	**Incubate the plates as follows:** • BA-C, CHO-C, TM-C: CO_2 incubator • MAC-O: O_2 incubator
4	Gram stain as per Gram-stain procedure MIC20115

12. PROCEDURE INSTRUCTIONS: MRO SCREENS

Step	Action
NOTE: Due to the 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	**Label the following media:** • DEN-O: Denim Blue agar ➢ For Nose/Groin screen: label half the DEN-O plate with the Nares label and the other half with the Groin label ➢ For other sites tested: label half a DEN-O plate • VRE-O: VRE agar ➢ Use whole plate
2	**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 for isolation  **Inoculate VRE agar:** • Inoculate the plate by pressing the swab firmly down on the slide to expel fluid. Rotate the swab several times to ensure all areas of the swab touch the slide. • Streak for isolation
3	Label the DEN-O plates with: R: (Date 1 day) and time incubated
4	**Incubate the plates as follows:** • DEN-O: O_2 incubator, not to exceed 24 hours, in MRSA racks • VRE-O: O_2 incubator, 48hours, in urine rack

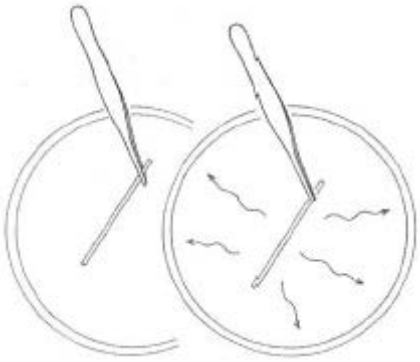
13. PROCEDURE INSTRUCTIONS: GROUP B SCREENS

Step	Action
1	**Label the following media:** • BHB-C: LIM Broth/Group B Broth: • BHBBS: Blood agar plate ➢ Place the plate in the "**SUBCULTURE PLATES**" rack located on the metal cart by the BSC
2	Break the swab off into the LIM broth. Recap loosely
3	**Incubate the media as follows:** • LIM Broth: CO_2 incubator
4	**After 18-24hr incubation:** • Remove GBS broth from incubator and subculture to the BAP labeled BHBBS located in the "**SUBCULTURE PLATES**" metal rack by the BSC • Incubate sub-cultured plate at 35ºC in CO_2 incubator for 18-24 hours

14. PROCEDURE INSTRUCTIONS: IUDs

Step	Action
1	**Label the following media:** • CMB-C: Cooked Meat broth • BRU-S: Brucella agar ➢ Place the plate in the "**SUBCULTURE PLATES**" rack located on the metal cart by the BSC
2	Using heat sterilized forceps – place IUD in cooked meat broth Label CMB broth: 10D: (10 days from inoculation date)
3	Incubate media as follows: • CMB-C: O_2 incubator, green rack labeled "THIOs"
4	**After 24hrs incubation:** • Subculture the CMB to Brucella agar (BRU-S) • Hold Broth for 10 days • Incubate BRU-S plate at 35ºC in an anaerobic tray with indicator and small pack for 10 days

15. PROCEDURE INSTRUCTIONS: CATHETER TIPS

Step	Action
1	Label the following plates: • BA-C: Blood agar • MAC-O: MacConkey agar
2	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.** 
3	**Incubate the inoculated plates as follows:** • BA-C: CO_2 incubator • MAC-O: O_2 incubator

16. PROCEDURE INSTRUCTIONS: ORAL CULTURES

Step	Action
1	Label the following: • SAB-R: Sabouraud dextrose agar
2	Inoculate plate by firmly rolling the swab over one-sixth of the agar surface and streak carefully for isolation • No smear is made Incubate SAB at room temperature for 48 hours

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	23 Mar 2017	Update to reflect new procedure changes	L. Steven