PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC10000 – Microbiology Specimen Handling	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
A guide to the handling of specimens submitted for bacterial culture including:

1. Description of media used
2. Gram stain smear labeling
3. Gram stain smear preparation
4. Media inoculation technique for swab and fluid specimens
5. Media inoculation technique for urine specimens
6. Setting up anaerobic jars for fluid and deep wound specimens

PURPOSE/RATIONALE:
This standard operating procedure describes the specimen handling of microbiology specimens processed in the microbiology laboratory at Stanton Territorial Hospital.

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

REAGENTS and/or MEDIA:

- Anaerobic KV agar (KV)
- Blood agar (BA)
- Brucella agar (BRU)
- Chocolate agar (CHO)
- Colistin-nalidixic acid agar (CNA)
- LIM broth (LIM)
- MacConkey agar (MAC)
- MRSA*Select* II agar (MRS)
- Sabouraud agar (SAB)
- 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 swabs
- Sterile pipettes
- Sterile swabs
- Anaerobic trays and jars
- Anaerobic indicators
- AnaeroGen packs
- AnaeroPouch packs
- Blood culture subculture vents

EQUIPMENT:

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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. Description of media used:

AGAR	TYPE	ATMOSPHERE	PUROSE
Anaerobic KV (KV)	• Selective	AnO_2-from all sources	• Inhibit gram-positive microorganisms and facultative anaerobic bacteria and select for gram-negative bacilli
Blood (BA)	• Enrichment	CO_2-wounds/fluids AnO_2-throats	• To grow most bacteria and determine the type of hemolysis. • Will not support *N.gonorrhoeae* or *Haemophilus* spp.

Brucella (BRU)	• Enrichment	AnO_2-from all sources	• To allow growth of all clinically significant anaerobes.
Chocolate (CHO)	• Enrichment	CO_2-from all sources	• To grow most bacteria, including *N.gonorrhoeae* and *Haemophilus* spp.
Colistin-nalidixic acid (CNA)	• Selective	CO_2-from all sources	• Inhibits most Gram-negative bacteria.
Laked Blood (KV)	• Selective • Enrichment	AnO_2-from all sources	• For the selective isolation of Gram-negative anaerobes
LIM (LIM)	• Selective • Enrichment	CO_2	• For the selective enrichment of group B S*treptococci*.
MacConkey (MAC)	• Selective • Differential	O_2-from all sources	• Gram-negative enteric agar that inhibits the growth of Gram-positive organisms and yeasts. • Differentiates lactose-positive organisms and lactose-negative organisms.
MRSA*Select*II (MRS)	• Selective • Differential	O_2	• For the selective and differential isolation of MRSA.
Sabouraud (SAB)	• Selective	Room temperature	• For the selective isolation of Yeast.
StrepB*Select* (GBS)	• Selective • Differential	O_2	• For the selective and differential isolation of GBS.
Thayer Martin (TM)	• Selective	CO_2-from all sources	• To select for *Neisseria gonorrhoeae* in mixed cultures.
Thioglycollate (THIO)	• Enrichment	O_2-from all sources	• To enrich the growth of anaerobic organisms.
Uri*Select* 4 (URI)	• Differential	O_2	• For the differential isolation of urinary tract pathogens.
VRE*Select* (VRE)	• Selective • Differential	O_2	• For the selective and differential isolation of VRE.

2. Gram stain smear labeling:

Swab specimen slide

Accession #
Patient Name
Specimen type

Fluid specimen slide

Accession #
Patient Name
Specimen type

Blood culture slide

Accession #
Patient Name
Bottle Type
(AE/AN/PE)

3. GRAM STAIN SMEAR PREPARATION:

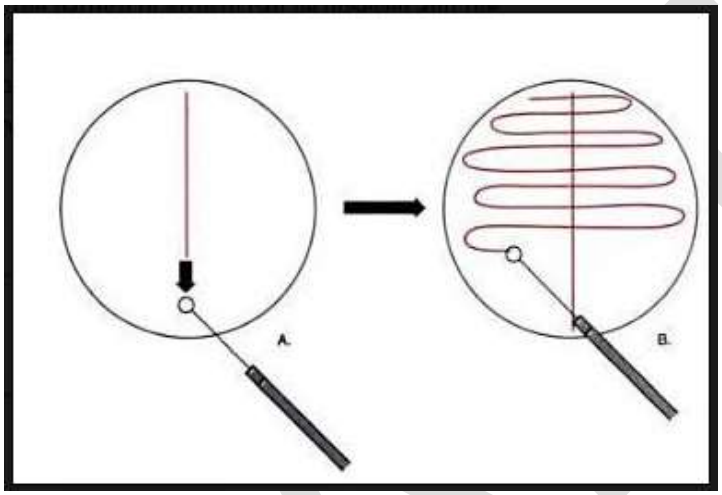
- For direct smears, prepare a monolayer of organism sufficiently dense for easy visualization but sparse enough to reveal characteristic arrangements. As a guideline, newspaper print should be visible through the smear
- Specimens received on swabs:
  - Roll the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
- Body fluid samples:
  - Use a sterile pipette to transfer 1 or 2 drops of the specimen directly to a sterile, ringed cytology slide
  - Spread the fluid with a disposable loop to obtain a thin smear
- CSF samples:
  - Use a sterile pipette to transfer 1 or 2 drops of the specimen directly to a sterile, ringed cytology slide
  - Allow the drop(s) to form one large drop, do not spread the fluid
- Blood Cultures:
  - Using the sub-culturing vent, apply 1 to 2 drops of the specimen directly to a sterile slide
  - Spread the blood with a disposable loop to obtain a thin smear
- Sputum specimens:
  - Select purulent or blood-tinged portions of sputum with a sterile swab
  - Spread the sample over a large area of the slide to form a thin film
- If there is insufficient specimen, omit the smear rather than the culture

4. MEDIA INOCULATION TECHNIQUE FOR SWAB AND FLUID SPEICMENS:

- Label media plates with LIS media labels.
- Touch specimen to one quadrant with swab or adding one to two drops of fluid with a sterile pipette.
- Streak with gentle pressure onto one-fourth to one-third of the culture plate using a sterile loop or needle, with a back and forth motion several times and without entering the area that was previously streaked. Avoid touching the sides of the media.
- Turn the plate a quarter turn. Pass the loop or needle through the edge of the first quadrant approximately three times, while streaking into the second quadrant. Continue streaking in the second quadrant without going back to the first quadrant.
- Rotate the plate another quarter turn and repeat the above procedure until one or two additional quadrants are streaked.

5. MEDIA INOCULATION TECHNIQUE FOR URINE SPECIMENS:

- Label media plate with LIS media label
- Using a disposable 1 µL calibrated loop, hold the loop vertically and immerse just below the surface of a well-mixed, un-centrifuged urine specimen. Avoid bubbles
- Deliver a loopful of well-mixed urine onto the Uri*Select* 4 plate and using the loop, make a straight line down the center of the plate and streak the urine by making a series of passes at 90° angles through the inoculum:



6. SETTING UP ANAEROBIC TRAYS AND JARS:

- ASAP after planting, set up anaerobic tray or jar except for throat specimens
- Place anaerobic plates into anaerobic tray or jar
- Tear open anaerobic indicator (kept in bucket with anaerobic packs) and withdraw wick 1 cm
- Place in small rectangle in anaerobic tray or jar
- Add AneroPouch pack to anaerobic tray or AnaeroGen pack to anaerobic jar
- Close the jar or tray
- For blood culture, fluid or deep wound specimens, label jar with 48 hour read date and place on bottom shelf of O_2 incubator labelled ANO_2 Jars/Trays
- For throat specimens, label with 24 hour read date and place on bottom shelf of O_2 incubator labelled 24 hour Throat Jars/Trays

LIMITATIONS:

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

CROSS-REFERENCES:

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

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) 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:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	10 Dec 19	Initial Release	L. Steven
2.0	30 Jan 22	Procedure reviewed and added to NTHSSA policy template	L. Steven