PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33100 – Wound Culture-Deep	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:
Deep wound cultures include the addition of anaerobic media for the detection of anaerobes. Anaerobic bacteria can cause a variety of infections including wound infections and a variety of abscesses. Anaerobic bacteria are overlooked unless the specimen is properly collected and handled. Anaerobes can vary in their sensitivity to oxygen and brief exposure to atmospheric oxygen is enough to kill organisms.

PURPOSE/RATIONALE:
To determine the presence or absence of bacterial pathogens in deep wound specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for deep wound culture.

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal Aspirate/Drainages/Pus • Clean, sterile container
Source	1. Deep wound specimens: • Bite, traumatic wound, third degree burn, deep surgical wounds, etc. 2. Superficial abscess specimens: • Boils, cyst, subcutaneous abscess, etc. 3. Deep abscess specimens: • Deep abscess, pus, etc.

Stability	If the sample is received in the laboratory and processed greater than 48 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Room temperature
Criteria for rejection	1. Unlabeled/mislabeled specimens 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

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Brucella agar (BRU) agar and Anaerobic KV agar (KV)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Anaerobic jar and pouch
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- Vitek 2 and supplies

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 Test Manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for deep wound culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, MAC, BRU and KV with the swab • Ensure all surfaces of swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • Prepare smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
2	Incubate all media: • Place BA and CHO in the CO_2 incubator • Place MAC in the O_2 incubator • Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with day 2 date and place in the O_2 incubator **NOTE:** Anaerobes should not be exposed to air for 42-48 hours after inoculation
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.

Probable Pathogens		
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • β-hemolytic streptococci • *Brucella**+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Chromobacterium* • *Erysipelothrix* • *Francisella**+	• *Haemophilus influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp. • Molds • *Moraxella catarrhalis* • *Neisseria gonorrhoeae* • *Neisseria meningitides**+ • *Nocardia* spp. • *Pasteurella multocida*	• *Pseudomonas aeruginosa* • *Salmonella* • *Shigella* • *Sphingobacterium* • *Staphylococcus aureus* • *Streptococcus anginosis* grp. • *Streptococcus pneumoniae* • *Vibrio* spp. • *Yersinia* spp.

Potential Pathogens	**Commensal Skin Flora**
• Aerobic gram-negative-bacilli not listed above • Anaerobes not listed above • *Enterococcus* spp. • *Staphylococcus lugdunensis* • *Staphylococcus intermedius* • Yeasts not listed above	• Coagulase-negative *Staphylococcus* • *Micrococcus* spp. • *Corynebacterium* spp. • *Bacillus* spp. not listed above • Nonpathogenic *Neisseria* spp. • viridans *Streptococcus* grp.

* Risk group 3 organism. If suspected, refer to Policy B-0160: "Specimens Containing Suspected Risk Group 3 Pathogens" for Primary Specimen Handling Flow Chart
+ All work-up should be performed in the BSC

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of aerobic growth in deep wound specimens	
1	Ensure growth on culture media correlates with gram stain results. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen
2	• Observe BA and CHO plates at 24 hours, 48 hours, and 72 hours • Observe MAC plate at 24 hours and 48 hours
3	**Single morphology growing on aerobic plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen or commensal skin flora</u>: ➢ Perform and report identification ➢ Perform and report susceptibility testing if any of the following are true: ○ 3 to 4+WBC in the gram stain ○ Organism is intracellular in the gram stain ○ Clinical diagnosis is infection ○ Patient is immunocompromised ○ Multiple cultures are positive for the same organism ➢ If NONE of the above are true, perform identification and list • <u>If organism is an anaerobe</u>: ➢ Refer to "Interpretation of anaerobic growth for deep wound specimens" portion of this procedure
4	**If multiple morphologies growing on plates:** • <u>If organisms are probable pathogens</u>: ➢ Perform and report identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organisms are potential pathogens</u>: ➢ Perform minimal identification and list if any of the following are true: ○ Moderate to numerous epithelial cells in the gram stain ○ No WBC in the gram stain ○ No clinical history that indicates infection was provided ○ ≥3 organisms growing, excluding probable pathogens • <u>If none of the above are true</u>: ➢ Perform and report identification of 1 or 2 predominant potential pathogens

	➢ Perform susceptibility testing and report if any of the following are true: ○ 3 to 4+WBC in the gram stain ○ Organism is intracellular in the gram stain ➢ Minimally identify and list any non-predominant potential pathogens ➢ Minimally identify and list >2 potential pathogens • <u>If organisms are commensal skin flora</u>: ➢ Minimally identify and list commensal skin flora

Step	Action
Interpretation of anaerobic growth for deep wound specimens	
1	• Observe BRU and KV plate at 48 hours and 5 days • If anaerobic growth is suspected, perform gram stain. If gram stain resembles growth on aerobic plates, further workup is not indicated. If growth does not resemble growth on aerobic plates, perform aerotolerance test. Refer to MIC53700-Aerotolerance Test **NOTE:** If specimen is from the neck or above, re-incubate BRU for a total of 10 days. Observe plates and broth at days 5, 8 and 10
2	**If single morphology growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU and KV for anaerobic growth • <u>If growth does not resemble growth on aerobic plates:</u> ➢ Perform and report identification ➢ Perform and refer to *Dyna*LIFE for susceptibility testing if ANY of the following are true: ○ Organism is a probable pathogen ○ Organism is intracellular or predominant in direct smear ○ Multiple or previous cultures are positive for the same organism ➢ If NONE of the above are true, perform identification and list organism
3	**If multiple morphologies growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU and KV for anaerobic growth • <u>If 2 anaerobes are isolated with or without aerobic growth</u>: ➢ List organisms based on gram stain identification • <u>If 2 anaerobes are isolated with aerobic growth or >2 anaerobes are isolated:</u> ➢ Report anaerobes as "Mixture of anaerobes"

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: "**No Growth after 1 Day. Further report to follow"**
No growth on aerobic media after 3 days	**INTERIM:** • Report: "**No growth aerobically after 3 days"** • Report: **"@Anaerobic culture to follow"**
No growth on anaerobic media after 5 days	**FINAL:** • Report: "**No anaerobes isolated after 5 days"**
No anaerobic growth after 5 days and specimen source is neck	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AC10**
Mix of skin flora	• Report: "**Mixture of skin flora"** • List quantitation
Mix of enteric Gram-negative bacilli	• Report: "**Mixture of coliform organisms"** • List quantitation
Growth or mix of other non-pathogenic organisms	• Report: "**Commensal flora"** or **"Commensal skin flora"** • List quantitation
Growth of >2 anaerobic organisms	• Report: "**Mixture of anaerobic organisms"** • List quantitation
Growth of 1-2 anaerobes with aerobic growth	• Report organism(s) identification • List quantitation
Growth of potential pathogen(s)	• Report organisms(s) identification • List quantitation • Report susceptibility as per interpretation of results
Growth of pathogen(s)	• Report organism(s) identification • List quantitation • Report susceptibility results as per ASTM
Pure growth of anaerobic organism	• Report organism identification • List quantitation • Report susceptibility as per interpretation of results

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to *Dyna*LIFE

LIMITATIONS:

1. The source of the specimen and how contaminated it may be with aerobic flora should influence the number and combination of primary isolation media used. For sterile sites that are considered sterile or if there are no organisms seen on the original gram-stained smear, an enriched non-selective medium such as Brucella agar is all that is required for anaerobic investigation. However, since many anaerobic infections are polymicrobial and mixed with facultative organims, a combination of selective and non-selective media is often required.
2. Anaerobic organisms may grow slowly and identification may take considerable time. It is important that the laboratory provid as much information as possible in an expeditious manner, through the use of preliminary reports.
3. Refer to MIC33000-Superficial Wound Culture for aerobic culture limitations.
4. The specimen must be obtained properly and transported ot the laboratory in a suitable anaerobic tranpsport container.
5. The technologist must perform aerotolerance testing on each isolate to ensure that it is an anaerobe.
6. A delay in processing of more than 1-2 hours may result in loss of recovery of strict anaerobes and the overgrowth of commensal microbiota.
7. A negative culture does not rule out an anaerobic infection.
8. False-negative cultures can result from contamination of the specimen with commensal microbiota or from prior antimicrobial therapy.
9. Inadequate specimen collection, improper speicmen handling and low organism levels in the specimen may yield a false negtive result.

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC20115-Gram Stain Procedure
- MIC33000-Superficial Wound Culture
- MIC34100-Body Fluid Culture for fluid specimens
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC36400-Referral of Category B Specimens to DL
- MIC53700-Aerotolerance Test

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	28 May18	Initial Release	L. Steven
2.0	30 Nov 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	01 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven