PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33000 – Wound Culture-Superficial	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 wide variety of microorganisms that reside on the skin and mucous membranes of the body, as well as those found in the environment, can cause skin and soft tissue infections. These organisms enter the body through breaks in the skin or mucous membranes, through wounds made by trauma or bites (exogenous), as a complication of surgery, foreign-body implants (endogenous) or they can be spread through the vascular system (hematogenous).

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

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

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal
Source	1. Superficial wound specimens: • Abrasion, cut, ulcer, impetigo, cellulitis, incision, 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 swabs 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) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- 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 superficial wound culture	
1	In the biosafety cabinet: • Inoculate BA and MAC 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 in the CO_2 incubator • Place MAC in the O_2 incubator
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
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 plate at 24 hours and 48 hours • Observe MAC plate at 24 hours
3	**If single morphology growing on plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen or commensal skin flora</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing if any of the following are true: o 3 to 4+WBC in the gram stain o Clinical diagnosis is infection o Patient is immunocompromised o Multiple cultures are positive for the same organism • <u>If organism is an anaerobe</u>**:** ➢ Perform and report full identification ➢ Perform and refer to *Dyna*LIFE for susceptibility testing if ANY of the following are true: o Organism is a probable pathogen o Organism is predominant in direct smear o Multiple or previous cultures are positive for the same organism
4	**If multiple morphologies growing on plates:** • <u>If organisms are probable pathogens</u>: ➢ Perform and report full 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: o Moderate to numerous epithelial cells in the gram stain o No WBC in the gram stain o No clinical history that indicates infection was provided o ≥3 organisms growing, excluding probable pathogens ➢ If none of the above are true: o Perform full identification and report 1 or 2 potential pathogens ➢ Perform susceptibility testing on potential pathogens and report if the following is true: o 3 to 4+WBC in the gram stain • <u>If organisms are commensal skin flora</u>: ➢ Minimally identify and list commensal skin flora

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day"** • Report: **"Further report to follow"**
No growth after 2 days	**FINAL:** • Report: "**No Growth after 2 Days"**
Mix of skin flora	• Report: "**Mixture of skin flora"** • List quantitation
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation
Mix of anaerobic organisms	• Report: **"Mixture of anaerobic organisms"** • List quantitation
Mix of non-pathogenic organisms	• Report: **"Commensal flora"** • List quantitation
Growth of potential pathogens where minimal identification and listing is required	• Report the minimal identification (i.e., Gram Negative Bacilli - Lactose Fermenter) • List quantitation
Growth of potential pathogen where full identification is required	• Report organisms full identification • List quantitation • Report susceptibility as per interpretation of results
Growth of pathogen	• Report organisms full identification • List quantitation • Report susceptibility results as per ASTM

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 results of wound cultures will only be as valuable as the quality of the specimen submitted, transport and expedient processing.
2. The microbiologist plays a critical role in the treatment of wound infections because practitioners often consider the report from the laboratory as definitive proof of infection. Providing inappropriate identifications and susceptibility results can prompt unnecessary treatment.

3. The presence of WBC is an indication of an inflammatory or infectious process, while the presence of epithelial cells indicates surface contamination of the specimen. Specimens containing numerous epithelial cells yield culture results of questionable accuracy in the diagnosis of the infectious process.
4. If a patient is immunocompromised or has poor vascular supply, inflammatory cells may not be present in the specimen as a guide to the extent of workup of the culture.
5. Antibiotics administered prior to sample collection may negatively affect the recovery of organisms associated with infection.
6. Many wound infections are polymicrobic and the isolation of an organism in culture may or may not correlate with infection of the wound.
7. Unusual diagnoses and treatment considerations may alter the usual policies of the laboratory in workup of organisms and reporting susceptibility results.
8. The lack of isolation of a pathogen does not necessarily mean that the laboratory was unable to detect the agent. Other inflammatory diseases can have the same presentations in infectious diseases, including the presence of WBC on the gram stain.

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC20115-Gram Stain Procedure
- 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

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:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	22 May 18	Initial Release	L. Steven
2.0	30 Nov 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	11 Jan 21	Procedure reviewed and added to NTHSSA policy template	L. Steven