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): NA	
Effective Date:	Next Review Date:
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved:
Accreditation Canada Applicable Standard: NA	

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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:
This standard operating procedure describes how to determine the significance of growth 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

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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), Columbia Naladixic Acid agar (CNA) 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 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 Test Manual for reagent quality control procedures

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PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for superficial wound culture	
1	In the biosafety cabinet: • Inoculate BA, CNA, and MAC with the swab • Ensure all surfaces of the 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 CNA 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^

GNB Aerobic:
- *Aeromonas* spp.
- *Brucella* spp.*+
- *Chromobacterium* spp.
- *Eikenella corrodens*
- *Pasteurella multocida*
- *Pseudomonas aeruginosa*
- *Salmonella* spp.
- *Shigella* spp.
- *Sphingobacterium* spp.
- *Vibrio* spp.
- *Yersinia* spp.

GNB Anaerobic:
- *Bacteroides fragilis*
- *Capnocytophaga* spp.

GNC/CB Aerobic:
- *Francisella tularensis**+
- *Haemophilus influenzae*
- *Kingella kingae*
- *Moraxella catarrhalis*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*

GPC Aerobic:
- β-hemolytic *Streptococci*
- *Staphylococcus aureus*
- *Streptococcus anginosis* grp.
- *Streptococcus pneumoniae*

GPB Aerobic:
- *Bacillus anthracis**+
- *Bacillus cereus*
- *Erysipelothrix* spp.
- *Listeria* spp.
- *Nocardia* spp.

GPB Anaerobic:
- *Actinomyces* spp.
- *Arcanobacterium* spp.
- *Clostridium perfringens*

Others:
- *Candida* spp.
- Molds

Potential Pathogens^	Commensal Flora
• Anaerobes not listed above • Enteric GNB not listed above • Non-enteric GNB not listed above • *Enterococcus* spp. • *Staphylococcus intermedius* • *Staphylococcus lugdunensis* • Yeasts not listed above	• *Bacillus* spp. not listed above • Coagulase-negative *Staphylococci* • *Corynebacterium* spp. • *Micrococcus* spp. • Non-pathogenic *Neisseria* spp. • viridans *Streptococcus* grp.

* Risk group 3 organisms. If suspected, refer to MIC40100-Suspect High Risk Organism Workup

+ All work-up should be performed in the BSC

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^ For organisms not listed, consult the Microbiology Technical Supervisor, or refer to the *Manual of Clinical Microbiology*

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 and CNA plates at 24 hours and 48 hours • Observe MAC plate at 24 hours
3	**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 flora</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing if ANY of the following are true: ○ 3 to 4+WBC in the gram stain ○ Clinical diagnosis is infection ○ Patient is immunocompromised ○ Multiple cultures are positive for the same organism • <u>If organism is an anaerobe</u>**:** ➢ Perform and report full identification ➢ Refer to APL for susceptibility testing if ANY of the following are true: ○ Organism is a probable pathogen ○ Organism is predominant in direct smear ○ Multiple or previous cultures are positive for the same organism
4	**Multiple morphologies 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</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 **NOTE:** Mixed enteric Gram-negative rods should be reported as mixture of coliform organisms, not reported individually **NOTE:** Mixed anaerobes should be reported as mixture of anaerobic organisms, not reported individually

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	➢ If none of the above are true: ○ Perform and report full identification ○ Perform and report susceptibility testing as per ASTM if ANY of the following are true: ❖3 to 4+WBC in the gram stain ❖Clinical diagnosis is infection ❖Patient is immunocompromised ❖Multiple cultures are positive for the same organism • <u>If organism is commensal flora</u>: ➢ Perform minimal identification and report as commensal flora **NOTE:** Mixed commensal flora should be reported as commensal flora, not reported individually and not reported as mixed

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"**
Growth of probable pathogen	• Report organism full identification • List quantitation • Report susceptibility results as per ASTM
Growth of potential pathogen or commensal flora where full identification is required	• Report organism full identification • List quantitation • If indicated by procedure, perform and report susceptibility testing as per ASTM
Growth of potential pathogen where minimal identification and listing is required	• Report the minimal identification (i.e., Gram Negative Bacilli - Lactose Fermenter) • List quantitation
Growth of commensal flora where minimal identification and listing is required	• Report: **"Commensal 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

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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 category B isolates to APL

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 the 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:

- 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
- MIC40100-Suspect High Risk Organism Workup
- LQM70620-Laboratory Critical Results List-Microbiology

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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	22 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	27 Feb 23	Procedure reviewed	L. Steven

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