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

SAMPLE INFORMATION:

Type	Swab • Amie's with or with charcoal
Source	1. Superficial wound specimens: • Abrasion, cut, laceration, ulcer, skin diseases (impetigo, folliculitis, cellulitis), first degree burn, superficial surgical incision, etc. 2. Superficial abscess specimens: • Boils, cyst, subcutaneous abscess, etc. 3. Drain specimens: • J-tubes, G-tubes, chest tube, abdominal, 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. Dry swabs. 4. Specimens for culture submitted in container with formalin. 5. 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) and Colistin Nalidixic Acid agar (CNA)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- 35° ambient air and 37° CO_2 incubators
- Wooden sticks
- 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.

- 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. 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 Test Manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for superficial wound culture	
1	In the biosafety cabinet, inoculate Blood agar and MacConkey agar from the specimen. Make gram stain. For chest tube drainage and tracheal swabs, add Chocolate agar.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place MAC plate in the O_2 incubator. Place BA plate and CHO plate, if applicable, in the CO_2 incubator.
4	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
5	Examine plates after 24 hour incubation. Record observations in the LIS.
6	Re-incubate CO_2 plate(s) for an additional 24 hours. Discard O_2 plate.
7	At 48 hours, examine plates and record observations.

Probable Pathogens		
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • β-hemolytic streptococci • *Brucella**+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Chromobacterium* • *Erysipelothrix* • *Francisella**+	• Molds • *Haemophilus influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp. • *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.

Possible 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 should be performed in the BSC.

INTERPRETATION OF RESULTS:

Step	Action
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Observe plates at 24 hours and 48 hours for growth. Count the number of types of organisms growing.
3	**Single morphology growing on plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform full identification and report all probable pathogens. ➢ Perform and report susceptibility testing as per ASTM. • <u>If organism is a possible pathogen or commensal skin flora</u>: ➢ Perform full identification and report all possible pathogens and commensal skin flora. ➢ Perform susceptibility testing and report if any of the following are true: o 3-4+WBC were seen in the gram stain o Organism is intracellular 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 gram stain and aerotolerance test. ➢ If aerotolerance is suggestive of anaerobic growth and it can be determined as a non-pathogen, report based on gram stain results. Freeze isolate. ➢ If aerotolerance is suggestive of anaerobic growth and cannot be determined to be a non-pathogen, report based on gram stain results and refer to DynaLIFE for further identification and susceptibility testing. Freeze isolate.

4	**Multiple morphologies growing on plates:** **NOTE: If selective media (CNA) was not inoculated and plates have large amount of growth, go back to specimen and inoculate selective media.** • <u>If organisms are probable pathogens</u>: ➢ Perform full identification and report all probable pathogens. ➢ Perform and report susceptibility testing as per ASTM. • <u>If organisms are possible 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 full identification and report 1 or 2 predominant possible pathogens. ➢ Perform susceptibility testing and report if any of the following are true: ○ 3-4+WBC were seen in the gram stain ○ Organism is intracellular in the gram stain ➢ Minimally identify and list any non-predominant possible pathogens. ➢ Minimally identify and list >2 possible pathogens. • <u>If organisms are commensal skin flora</u>: ➢ Minimally identify and list commensal skin flora.

REPORTING RESULTS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day. 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.
Growth or mix of other non-pathogenic organisms	• Report **"Commensal flora"** or **"Commensal skin flora"** • List quantitation.
Growth of potential pathogen(s) where minimal identification and listing is required	• Report the minimal identification under the isolates tab (i.e. Gram Negative Bacilli - Lactose Fermenter). • List quantitation.
Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM. • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location. **NOTE:** If the same patient has the same pathogen isolated in different specimens, do not perform susceptibility testing for each specimen. Perform for the first specimen and refer subsequent specimens for up to 5 days.

LIMITATIONS:

1. 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.
2. The results of wound cultures will only be as valuable as the quality of the specimen submitted, transport and expedient processing.
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 disease can have the same presentations in infectious diseases, including the presence of WBC on the gram stain.

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		Initial Release	L. Steven