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

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal Aspirate/Drainages/Pus • Clean, sterile container
Source	1. Deep wound specimens: • Bite (animal or human), traumatic wound (gunshot, stabbing), 3rd degree burn, deep surgical wounds, etc. 2. 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 have compromised the recovery of organism"
Storage Requirements	Do not refrigerate or incubate before or during transport. If there is a delay, keep sample at room temperature as at a lower temperature there is likely to be more dissolved oxygen, which could be detrimental to anaerobes.
Criteria for rejection	1. Unlabeled/mislabeled specimens. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs. 4. Specimens for culture submitted in container with formalin.

NOTE:

- **Refer to MIC34100 – Body Fluid Culture for fluid specimens.**
- **Refer prosthetic device specimens for culture to DynaLIFE.**
- **Refer tissue or biopsy specimens for culture to DynaLIFE.**

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Colistin Nalidixic Acid agar (CNA), Brucella agar (BRU) and Brucella Laked Blood agar with Kanamycin and Vancomycin (KV)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- Anaerobic jar and pouch
- 35° ambient air and 35° 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 deep wound culture	
1	In the biosafety cabinet, inoculate Blood agar, Chocolate agar, MacConkey agar, Brucella agar and Brucella Laked Blood agar with Kanamycin and Vancomycin from the specimen. Make gram stain.
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 and CHO plates in the CO_2 incubator.
4	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation.
5	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
6	Examine aerobic plates after 24 hour incubation. Record observations in the LIS.
7	Re-incubate CO_2 plates for an additional 48 hours. Re-incubate O_2 plate for an additional 24 hours.
8	At 48 hours, examine plates and record observations in the LIS.
9	At 72 hours, examine plates and record observations in the LIS.
10	Examine anaerobic plates after 48 hours incubation and record observations in the LIS. Re-incubate BRU anaerobically for an additional 72 hours. If specimen is from the neck or above, re-incubate BRU for an additional 8 days. After 5 or 8 days, as applicable, examine plate and record observations in the LIS.

Probable Pathogens		
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • β-hemolytic streptococci • *Brucella* spp.*+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Chromobacterium* • *Erysipelothrix* • *Francisella**+	• Molds • *Haemophilus influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp. • *Moraxella catarrhalis* • *Neisseria gonorrhoeae* • *Neisseria meningitidis**+ • *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
Interpretation of aerobic growth of deep wound specimens	
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 aerobic plates at 24 hours, 48 hours and 72 hours for growth. Count the number of types of organisms growing.
3	**Single morphology growing on aerobic plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform full identification and report. ➢ 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. ➢ 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>: ➢ Refer to “Interpretation of anaerobic growth for deep wound specimens” portion of this procedure.

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 and organisms possible pathogens</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.

Step	Action
Interpretation of anaerobic growth for deep wound specimens	
1	Observe anaerobic plates at 48 hours. Examine under stereoscope and record observations in the LIS. Mixed cultures are time consuming, add increased cost to testing and may result in misidentification.
2	Identification of anaerobic growth should start with careful examination of colonial morphology and gram stain results.
3	Aerotolerance test should be performed on all anaerobes. Refer to MIC51700 – Aerotolerance Test.
4	**Single morphology growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth. • <u>If growth does not resemble growth on aerobic plates:</u> ➢ Perform gram stain and aerotolerance test. ➢ If aerotolerance is suggestive of anaerobic growth and this organism combined with aerobic growth result in ≥ 3 organisms growing, excluding pathogens, report organism based on gram stain identification (i.e. anaerobic gram-negative bacilli). ➢ If aerotolerance is suggestive of anaerobic growth and this organism combined with aerobic growth result in <3 organisms growing, excluding pathogens, report organism based on gram stain results and refer to DynaLIFE for further identification and susceptibility testing.
5	**Multiple morphologies growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth. • <u>If 2 anaerobes are isolated and no aerobic growth is present</u>, ➢ List organisms based on gram stain identification (i.e. anaerobic gram-negative bacilli). • <u>If 2 anaerobes are isolated with aerobic growth or > 2 anaerobes are isolated:</u> ➢ Report anaerobes as “Mixture of anaerobes”.

REPORTING RESULTS:

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 growth on anaerobic media after 5 days and specimen source is from above the neck	**FINAL:** • Report: "**No anaerobes isolated after 5 days"** • Add test comment **}AC10** to state: **"The anaerobic culture will be incubated for an additional 5 days for the isolation of Actinomyces spp. A further report will follow only if positive".**
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) based on gram stain identification in the isolates tab. • 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.
Pure growth of anaerobic organism	• Report organism based on gram stain results under the isolates tab. • List quantitation. • Add isolate comment **&REF4** to state: **"This organism has been referred for further identification and susceptibility testing."** • Refer organism to DynaLIFE for identification and susceptibility testing as per MIC10510 – Referral of Category B Specimens to DynaLIFE. • Use anaerobic transport media. • Freeze isolate and log into stored isolates binder.

CULTURE NOTES:

- 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 organims 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.
- Anaerobic organisms may grow slowly and identification may take considerable time. It is important that the laboratory provid as much inforation as possible in an expeditious manner, through the use of preliminary reports.

LIMITATIONS:

1. Refer to MIC333000-Superficial Wound Culture for aerobic culture limitations.
2. The specimen must be obtained properly and transported ot the laboratory in a suitable anaerobic tranpsport container.
3. The laboratory must be able to verify that the anaerobic enivronment is indeed anaerobic.
4. The technologist must perform aerotolerance testing on each isolate to ensure that it is an anaerobe.
5. 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.
6. A negative culture does not rule out an anaerobic infection.
7. False-negative cultures can result from contamination of the specimen with commensal microbiota or from prior antimicrobial therapy.
8. Inadequate specimen collection, improper speicmen handling and low organism levels in the specimen may yield a false negtive result.

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