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| Skin and Superficial Wound Culture |
| **Purpose** | This procedure provides instruction for Skin and Superficial Wound Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | The skin is the most accessible organ of the body, the one most easily traumatized and therefore the one subjected most frequently to the risk of infection. Skin serves as a physical and chemical barrier to microorganisms. The acellular, outermost layer of skin and tightly packed cellular layers underneath provides an impenetrable physical barrier to all microorganisms, unless damaged. Skin infections can be caused by a variety of organisms from the external environment through breaks in the skin or from organisms that reach the skin through the blood as part of a systemic disease. Because virulence factors are not always necessary, virtually any species can be involved. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | SKIC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 3% hydrogen peroxide
* Gram Stain reagents
* Oxidase reagent
* Staphaurex™
* Other supplies as necessary for the identification of common agents
 | * Glass slide (GMST)
* Sterile disposable pipette
* Sterile tube (s)
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
* Vortex mixer
 | Refer to the Sunquestspecimen label for media information.* Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Saline, Normal 1 mL (SLNE)
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| **Specimen** | 1. Acceptable specimens
* Exudates or aspirated material from lesion
* Skin scraping
* Aspirated material is superior to a swab specimen. If a swab must be used, collect two, one for culture and one for Gram stain.
1. SDES codes/Specimen type
* State anatomic location of specimen.

Refer to [Lab Test Directory – Skin/Superficial Lesion Culture and Gram Stain](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033285.asp). |
| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
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| **Procedure** | InoculationWarm all media before inoculation. Label all plates and slides properly with the patients name, accession number and date. 1. Inoculate the media in the order of the least selective first to prevent carryover of inhibitory substances to another medium. Refer to the Sunquest specimen label for the order of inoculation.

Specimen processingAspirates and exudatesIf specimen is received in a syringe, transfer the entire amount into a sterile tube and mix well. Vortex on 5 or 6.If the specimen is received in a syringe and the volume is small, rinse syringe with a small amount of SLNE to remove the specimen from the syringe. Mix well.Place 1-2 drops directly on each plate and onto a slide.Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.Specimens received on swabs1. Emulsify swab in 1.0 ml of SLNE by vortexing well. Squeeze the swab against the side of the tube to express remaining fluid and then discard.
2. Place 1-2 drops of the suspension directly on each plate and onto a slide.
3. Streak plates semi-quantitatively for primary isolation.
4. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
5. Pass the loop back and forth through the inoculum in the first quadrant several times.
6. Flame the loop, turn the plate a quarter turn and pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.
7. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 4-5 times.

~AUT00291. **Incubation**
2. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC
3. Place MAC in ambient air incubator at 35ºC.
4. **Gram stain examination**

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
2. Blot excess oil from slide. Hold slide for one week.
3. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
4. **Culture examination:** Examine plates daily for 4 days.
5. Day 1:
6. Examine primary plates.
7. Plated media
8. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
9. Correlate colony types with the direct Gram stain.
10. Use the initial Gram stain to help determine the extent of work-up required on the culture. The presence of many WBCs indicates an infectious process. Squamous epithelial cells represent surface contamination and the isolate work-up should be minimal.
11. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
12. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
13. Subculture organisms that are not well isolated to appropriate media for further work-up.
14. Re-incubate primary plates and subcultures for an additional day.
15. Report preliminary results.
16. Day 2
17. Examine primary plates from the previous day for additional microorganisms.
18. Read and record identification tests and susceptibilities from the previous day.
19. Set up additional tests as needed.
20. Send updated or final report.
21. Call MRSA results to patient’s caregiver, if not an E.R. culture. Freeze for future reference.
22. Additional Days
23. Complete identification and susceptibility testing procedures until all significant isolates are finished.
24. Send updated report and finalize.
25. If there is no growth on the plates, discard at 4 days and final the report as “No Growth, 4 days”.
26. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.
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| **Method Performance Specifications** | * 1. Perform definitive identification and susceptibility testing on the following:
1. Any quantity of a **probable pathogen**, i.e., *S. aureus, Ps. aeruginosa,* etc.
2. **Potential pathogens** with unpredictable susceptibility patterns such as the *Enterobacteriaceae.*
3. Organisms isolated from **patients with catheter-related infections**.
4. **Organisms isolated in pure culture** and also observed in the specimen Gram stain.
	1. Perform limited identification and no susceptibility testing on the following:
5. Use Sunquest code **USF, “**Usual skin flora”, in reporting mixed cultures with 3 or more organisms representing skin colonization or contamination. Probable skin contaminants include:

Coagulase-negative staphylococci Diphtheroids *(Corynebacterium ssp.)*Viridans streptococci *Bacillus* sp. with many epithelial cells / no PMNSEnterococci1. Isolates from sources such as decubitis ulcer, perianal abscess or fistula and intestinal drainage: If the culture grows more than 3 organisms, none which is predominant, report as “MIXED FLORA, no further identification” (**MF**). Hold the plates for further testing if requested. Susceptibility testing is not indicated in specimens that are contaminated with bowel contents. Broad spectrum coverage is generally used for normal intestinal flora.
2. Consult the physician on questionable cultures.
3. Perform identifications and susceptibilities if requested by the physician.
	1. Rule out *Corynebacterium diphtheriae* if cutaneous diphtheria is suspected. Hold plates 5 days.
	2. Rule out *Corynebacterium jeikeium* (group JK) from catheter-related wounds.
	3. Perform definitive identification and susceptibilities on *H. influenzae* and *S. pneumoniae* if the diagnosis is periorbital cellulitis.
	4. Identify organisms associated with bite wounds, i.e., *Eikenella corrodens, Pasteurella* sp., *Capnocytophaga canimorsus* (DF-2), EF-4, *Staphylococcus intermedius, Weeksella zoohelcum,* etc.
	5. See procedures in [MCVI](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.60%20BioTerrorism%20Protocol.docx) 3.60 Bioterrorism Protocol for specific LRN Bioterrorism protocols if BT organisms are suspected.
	6. If possible *Bacillus anthracis*, *Brucella, Burkholderia mallei, Francisella tularensis, or Yersinia pestis:* do only preliminary testing. All manipulations need to be performed in a class II BSC hood, and plates sealed. Call MDH and send isolate if a BT organism cannot be ruled out. These organisms are highly contagious and pathogenic. Do not use automated ID systems.
	7. Occasionally, *Mycobacterium chelonae* and *M. fortuitum* may cause infections. These organisms may be misidentified as diphtheroids, especially in broth culture.
	8. Culture of superficial wounds for anaerobes in the absence of clinical indication is expensive and unrewarding.
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| **Result Reporting** | 1. Culture results: Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semi-quantitatively, i.e., 1+, 2+, 3+ or 4+.

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| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

1. No growth cultures: Update culture status in the Observation result box (*Culture Entry* tab), by using the “No Growth” update key (‘). Report as “No growth “*x*” days". Final ( / ) culture at 4 days.
2. Positive cultures:

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to followWorkups: Wkup # 1 Workup Components Med : SB GMS : STPH Desc : BH SC : SB Id : SAUR SLC : POS VMIC : 1 MSID :1 1. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Document date and time called in computer. Freeze isolate for future reference.

1. 3+ METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\*2. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.3. \*\*Called to Linda S., RN L8 @ 1300 7/7/031. If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to policy [MCVI 5.1 Labeling Errors-Specimen Mix-up](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.1%20Labeling%20Errors-Specimen%20Mix-up.docx) for Sunquest report entry information.
2. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code **SRPT** (supplementary report) must be used in SREQ or *Culture Observations* as follows:
* Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.
* Requests for additional testing: In the *Misc. Updates* tab, enter SRPT in SREQ followed by the request.
* Refinal the culture when identifications and/or testing are complete.
1. Gram stains: Report Gram stain results by selecting the *Direct Exam* tab. Follow [MC 2.0 Gram stain](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%202%20Staining%5CMC%202.0%20Gram%20stain.docx) procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI 2. 4+ WBC'S1. Review **Culture Summary** for accuracy before filing report.
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| **References** | 1. Leber, Amy, 4th edition Aerobic bacteriology, *Clinical Microbiology Procedures Handbook*, 2016, American Society for Microbiology, Section 3.13.1, 3.3.2, Washington, D.C.
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| **Appendices** | BATTERY: SKICSPEC MEDIA0 SLNE,CHOC,SB,CNA,MAC,GMST |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 08/08/1981 | Initial Version |
| 1.1 | Pat Ackerman | 02/03/1992 |  |
| 1.2 | Pat Ackerman | 07/30/2003 |  |
|  | 1.3 | Pat Ackerman | 12/17/2004 |  |  |  |
| 1.4 | Pat Ackerman | 01/22/2006 |  |
| 1.5 | Pat Ackerman | 08/20/2007 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. |
|  | 1.6 | Jessica Craig | 06/10/2010 | Updated into online format. |
|  | 2 | Becky Carlson | 4/18/2015 | Re-numbered from MC 425 for CMS load. |
|  | 3 | Susan DeMeyere | 9/7/2017 | Changed reporting to keep culture open while THIO is incubating.  |
|  | 3 | Susan DeMeyere | 10/24/2018 | Biennial review |
|  | 4 | Susan DeMeyere | 10/9/2020 | Removed use of and reporting results from THIO media. |
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