KETTERING Health Network

Aerobic Culture

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Document Control Number: MB.20.020.00

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Retired: Date:

I. 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 **(Table 1).** These organisms enter the body through breaks in the skin or mucous membranes, through wounds made by trauma or bites, or as a complication of surgery or foreign-body implants, or they can be spread to the tissues through the vascular system.

The workup of wound cultures is dependent on the specimen source and the quality of the specimen, as indicated by the ratio of white blood cells (WBCs or PMNs) to squamous epithelial cells (Epis) seen on the primary gram stain. Workup is also dependent on the number of organisms present and their potential as pathogens. Occasionally, consultation with the clinician may be warranted to aid in determining the appropriateness of workup of an organism growing in a culture.

II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING

A. **Specimens**
   1. **For complete specimen requirements, refer to the Laboratory Collection Manual.**
   2. **For complete specimen processing requirements, refer to the Specimen Processing Procedure.**

B. **Transport and Handling**

C. **Rejection Criteria**
   1. Do not accept specimens received with formalin.
   2. Do not accept specimens with needle still attached. Call nurse and have them come remove it before processing. Make note to specimen when this occurs.
   3. For multiple requests (acid-fast bacilli, fungal, bacterial, and viral) but little specimen, contact the physician to determine which assays are most important and reject the others as "Quantity not sufficient."

III. PROCEDURE

A. The Gram stain is used to ensure the quality of specimen and to evaluate workup.
   1. White blood cells (WBCs) in the absence of squamous epithelial cells (SEC) clearly indicates inflammation and signals a good specimen. Quantitate the presence or absence of WBC's, epithelial cells if present, and bacteria and fungi. Document NOS (no organism seen) if bacteria or yeast is absent.
   2. The presence of SEC signals contamination with commensal flora and should be noted in the report. If WBCs and SECs are both noted in the smear, add a comment to the report **.FLORA** "Squamous epithelial cells seen on Gram stain indicate that interpretation may be compromised due to the presence of normal skin flora. Interpret culture results with caution."
   3. SECs with no WBCs indicates little or no inflammation (in immunocompetent patients) and should be rejected if possible. Otherwise, workup the specimen and add a comment to the report **.FLORA**
   4. In general, if WBCs outnumber SECs per low power field, continue with routine workup.

B. **Sterile Sites: Tissues / Aspirates**
   1. **Solid media** should be incubated for 3 days in the CO_2 incubator.
      a. Plates should be incubated for 18-24 hours before a preliminary report is given and read again every 24 hours.

b. Perform identification and AST on up to 2 predominant pathogens, excluding skin flora. Report minimal identification of non-predominant pathogens and skin microbiota. (See Table 1. Aerobic and anaerobic isolates from acute and chronic infections.) If there are three or more different aerobic colony types with no predominant organism, the culture can be reported as "MIX" (Mixture of ≥3 Organisms) and finalize after 3 days of incubation.
c. If there are five or more different colony types, either aerobes or anaerobes, with no predominant organism, the aerobic culture can be finalized out after three days of incubation as "MIX5" (Mixture of ≥5 organisms).

2. **Enriched Thioglycolate broth (Thio)** should be incubated for 5 days in the non-CO_2 incubator.
   a. Subculture the THIO broth to an aerobic BAP and CHOC if it becomes cloudy and there is no growth on the primary culture plates or if the growth on the culture does not match the gram stain. A BRUCELLA incubated anaerobically can be added if an anaerobe is suspected.
      1) Add workup "Thio" to culture.
      2) Record growth or no growth at 24 and 48 hours as appropriate using the pull-down menu associated with each subcultured media.
   b. If an organism is isolated from broth only, no quantitation can be made. Report using comment "Broth" (Isolated from broth only, unable to quantitate)
   c. If the primary culture plates are growing, hold the Thio until the culture plates are final.

C. **Non-Sterile Body Sites: Superficial Wounds / Skin Sites / Eye Cultures should** be incubated for 3 days in the CO2 incubator.
   1. Semi-quantitate the growth and enter each isolate by name (e.g. GNB, Staph), in Beaker.
   2. Identify up to 2 predominating pathogens, excluding skin flora. Perform ID and AST as appropriate. Indicate the type and amount of non-predominating organisms with descriptive ID only.
      a. When pure or predominant, ID and AST should be performed (as appropriate for each organism on the following organisms:
         1) *Staphylococcus aureus* - always work up, no matter the quantity.
         2) Possible *S. lugdunensis*- usually hemolytic, sticky, yellow or tan, PYR positive. Identify organism. If not *S. lugdunensis* report as Coagulase-negative *Staphylococci* or consider part of normal flora.
         3) *Streptococcus pyogenes* (Group A*)* – always work up, no matter the quantity.
         4) Enteric Gram-Negative Rods – If pure, or if it is one of two pathogens present perform ID and AST. If not predominant, list as Gram Negative Rod, add comment Lactose Fermenter or Non-Lactose Fermenter and NFW. If more than 2 enteric Gram-negative rods are present, then list organisms and add comment **"Three or more morphologies are present. Quality of specimen may be compromised"**
         5) Non-*Enterobacteriaceae* gram negative rods- Identify and perform AST on any Gram-negative organism that does not grow on MAC, such as *Pasturella* species. Identify *P. aeruginosa* and *S.maltophilia*. Perform AST if pure, or one of two predominant pathogens. If not pure or predominating, list and add NFW. All other Non-

Enterobacteriaceae identify and perform AST only if one of two predominant pathogens.

6) *Streptococcus pneumoniae*- perform ID and AST if pure and one of two predominant organisms. If not pure or predominating, report ID with NFW.
7) *Enterococcus species* – Identify and perform AST if pure or a predominant pathogen. Include as normal flora if specimen is a swab, mixed, or not one of two predominating pathogens.
8) *Neisseria* species, rule out gonorrhoeae on eye specimens

b. If pure or predominant from a non-sterile body site, the following organisms should be indicated with descriptive identification only:
   1) Coagulase-negative *Staphylococci* (CNS) – perform ID and AST only if it is pure and from a tissue or aspirate specimen, **and** PMN's are seen in the gram stain; or if it is pure and isolated from multiple cultures. Report Coagulase-negative *Staphylococci* and add NFW if in mixed cultures from sterile sites. Include in normal flora if in mixed cultures, and from a non-sterile site.
   2) Gram positive rods – If culture is pure, predominant or from a sterile site perform ID. If not predominant or from non-sterile site, include with normal flora. *Bacillus anthracis, Arcanobacterium, C.diphtheriae, C. ulcerans* should be ruled out if pure, predominant or growing in multiple cultures.
   3) Beta Hemolytic Strep not *Streptococcus pyogenes* - ID only.
   4) Viridans and other non-hemolytic Streptococci- work up only if pure and from a sterile site, aspirate or tissue culture. Minimum ID if predominant or pure from a swab/non-sterile site. Include in normal skin flora if culture is mixed and not predominate.
   5) *Moraxella species*
   6) *Neisseria species* (rule out GC when applicable [anal wound, etc.])
   7) Yeast – Check for fungal culture, refer if possible. Perform ID if pure or predominant and from a sterile site. If from a non-sterile site, or from a sterile site but not predominate, list as YEAST and add No Further Work Up (NFW). Rule out *Cryptococcus neoformans*. Yeast is only normal flora from an oral or fecal site and must be less than or equal to the flora present.
   8) Mold- tape plates and give isolate to mycologist to identify.

c. In the presence of a predominant organism, a mixture of three or more of these organisms in equal amounts should be called "mixed flora isolated". (For example: A culture with many *S. aureus*, few Corynebacterium, few Alpha Strep, and few CNS would be reported as: "Many *S. aureus* isolated and few mixed flora isolated.") Mixed flora includes a MIX of *diphtheroids, Lactobacillus, Micrococcus, and* coagulase negative *Staphylococcus*. Normal oral flora includes a MIX of alpha hemolytic strep including *enterococcus and pneumococcus, Neisseria spp., diphtheroids, Lactobacillus, Micrococcus*, and coagulase negative *Staphylococcus*.

d. Identify any number of microorganisms that grow only on CHOC, and not BAP (*N.gonorrhoeae*, *Hemophilus*, and *Francisella*). Any organism that grows on CHOC only or is suspected of *N. meningitidis* must have all testing performed under a biosafety hood.

e. If there are > 3 colony types with no predominating organism(s), the culture can be reported as "mixture of >= 3 organisms

3. Ear cultures:

a. Identification and AST should be performed on any amount of *S. pneumoniae* isolated.
b. Any amount of yeast or mold should be identified and reported.
c. When pure or predominant, identification should be performed on *Haemophilus* species.
d. Work up all other pathogens if they are one of two predominant organisms.

4. Genital Cultures:
   a. When pure or predominant, identification and AST (as appropriate for each organism) should be performed on the following organisms:
      1) *Staphylococcus aureus*
      2) *Streptococcus pyogenes* (Group A)
      3) *Streptococcus agalactiae* (Group B)
      4) *Neisseria gonorrhoeae*
   b. When pure or predominant, all other organisms should be listed with descriptive identification only.
   c. If there are ≥3 types of non-predominating organisms, they may be quantitated and reported as "MIX" (Mixture of ≥3 Organisms)
5. Catheter Tips
   a. Solid media should be incubated for 3 days and examine for growth every 24 hours.
   b. >=15 colonies perform ID and AST.
   c. <15 descriptive ID only.

IV. INTERPRETATION

A. Reporting selected organisms in mixed cultures can lead to erroneous interpretation of the number and variety of infecting pathogens.
B. Performance of AST is not indicated in cases of mixed flora indicative of infection of the abdominal cavity with bowel contents. Treatment should include broad-spectrum coverage for normal intestinal microbiota.
C. Use of the Gram stain can improve the accuracy of evaluating the importance of each potential pathogen. Organisms present in the Gram stain of an appropriately collected specimen correlate with ≥10^5 organisms per g of tissue.
D. Clinical studies have demonstrated that the microbial load in an acute wound can predict delayed healing or infection. In general, the more numerous the organisms, the more likely they are to be indicative of infection.

V. REPORTING RESULTS

A. Report the gram stain. Refer to the gram stain procedure.
B. Negative cultures, prelim report using the following codes. Final report as NG72

Time Incubated	Code
18-24 hours	"NG24" (No Growth at 18-24 Hours)
48 hours	"NG48" (No Growth at 48 Hours)
72 hours	"NG72" (No Growth at 72 Hours)
4 days	"NG4D" (No Growth at 4 Days)
5 days	"NG5D" (No Growth at 5 Days)

C. Positive cultures - quantitate growth using the following codes.

Number of Colonies	Code
0-20 colonies	Rare
20 or more colonies in the 1st and 2nd quadrants	Light
Growth out to the 3rd quadrant	Moderate
Growth out to the 4th quadrant	Heavy

VI. CRITICAL DETERMINANTS

A. 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.
B. The results of wound, abscess, and tissue cultures will only be as valuable as the quality of the specimen submitted, transport, and expedient processing.
C. The presence of PMNs 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, and one can consider rejection of these specimens for culture.
D. Antibiotics administered prior to sample collection may negatively affect the recovery of organisms associated with infection.

VII. REFERENCES

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Table 1. Commonly isolated aerobic and anaerobic isolates from acute and chronic infections.

Aerobic and facultative microorganisms	Anaerobic bacteria	Aerobic microorganisms from unusual, specialized, and zoonotic infections	Yeasts
Coagulase-negative staphylococci	*Finegoldia magna*	*Aggregatibacter actinomycetemcomitans*	*Candida albicans*
Staphylococcus aureus	*Peptostreptococcus spp.*	*Aeromonoas spp.*	*Candida krusei*
Beta-hemolytic streptococci	*Peptoniphilus spp.*	*Bacillus anthracis*	*Candida parapsilosis*
Enterococcus spp.	*Actinomyces spp.*	*Bergeyella zoohelcum*	
Streptococcus spp. (viridans group)	*Clostridium spp.*	*Capnocytophaga spp.*	
Arcanobacterium hemolyticum	*Eggerthellas spp.*	*Neisseria animoralis*	
Corynebacterium spp.	*Eubacterium limosum*	*Chromobacterium violaceum*	
Bacillus cereus	*Propionibacterium acnes*	*Eikenella corrodens*	
Escherichia coli	*Bacteroides fragilis group*	*Erysipelothrix rhusiopathiae*	
Serratia spp.	*Prevotella spp.*	*Francisella tularensis*	
Klebsiella spp.	*Porphyromonas asaccharolytica*	*Haemophilus spp.*	
Enterobacter spp.	*Fusobacterium necrophorum*	*Kingella kingae*	
Citrobacter spp.	*Veillonella spp.*	*Nocardia spp.*	
Morganella morganii		*Pasteurella multocida*	
Providencia stuartii		*Streptobacillus moniliformis*	
Proteus spp.		*Vibrio vulnificus*	
Acinetobacter baumannii			
Pseudomonas aeruginosa			
Stenotrophomonas maltophilia			
Sphingobacterium multivorum			

Table 2. Guideline for organism work- up

Situation	Organism	Action
Pure or 1 or 2 predominant pathogens from a sterile site (TISSUE/ASPIRATE)	*1. Staphylococcus aureus (in any amount)* *2. S.lugdunensis* *3. Gram negative rods* *4. Streptococcus pyogenes* *5. Streptococcus pneumoniae* *6. Enterococcus spp.* *7. CNS (only if pure AND WBCs are seen in GS)* *8. Viridans/Non-heme Strep (ID only, MIC upon request)* *9. Yeast*	Perform ID/AST
Pure of 1 of 2 predominant pathogen from a non-sterile site (SWAB/SKIN/EYE)	*1. Staphylococcus aureus (in any amount)* *2. S.lugdunensis* *3. Gram negative rods* *4. Streptococcus pneumoniae* 5. *Enterococcus spp.*	Perform ID/AST
Sterile Site – Not Predominant	1. Gram negative rods 2. *Streptococcus pneumoniae* 3. Gram positive rods 4. Beta Hemolytic Strep not *Streptococcus pyogenes* 5. Viridans/Non-heme Strep 6. *Moraxella spp.* 7. *Neisseria* spp. (must rule out *gonorrhoeae* if EYE culture) 8. Yeast	ID only, NFW
Non-Sterile Site – Not Predominant	1. CNS 2. *Enterococcus spp.* 3. Viridans/non-hem Strep 4. Gram positive rods 5. Yeast (Only from oral or fecal sites. Any other site - ID)	Include in Mixed Flora Isolated