HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Tissues Culture		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and set responsibilities for processing tissues Culture.

2. DEFINITONS:

2.1 N/A

3. POLICY:

3.1 Surgical biopsies should be considered sterile specimens and therefore the isolation of any organism(s) should be considered significant.
3.2 Tissue should be collected in a clean, sterile container with a small amount of sterile saline.
3.3 If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

4. PROCEDURE:

4.1 **Processing of Specimen:**

4.1.1 If possible, macerate the tissue using a grinder (small tissue sample) or a scalpel (large tissue sample). Otherwise, add sterile broth or saline to the sample, incubate at 35+2 °C for 2 hours then inoculate the media.

4.1.2 Bone should be inoculated directly into Fastidious Anaerobic Broth (if available) and is not macerated.

4.1.3 Direct examination: Using clean, sterile slides make touch preparation smears from a cut surface of the biopsy, Gram stain and examine for presence of organisms and pus cells.

4.1.4 Fungus culture is not set up for wound debridement tissue, joint capsules, gas gangrene tissue and necrotizing fasciitis tissue unless specifically requested.

4.1.5 Inoculate the following media with the sample:

Media:	Incubation:
Blood Agar (BA)	O2,35+2 °C x 48 hours
MacConkey Agar (MAC)	O2,35+2 °C x 48 hours
Chocolate Agar (CHOC)	CO2,35+2 °C x 48 hours
Blood/ MacConkey's agar	anaerobic ,35+2 °C x 48 hours.
Fastidious Anaerobic Broth (THIO)*	O2, 35+2 °C x 48 hours
Sabauroud Agar (SD)	O2, 30°C x 3 weeks

*If available

4.1.6 If organisms were seen on direct Gram stain and culture yields no growth, check original Gram stain and re-incubate all aerobic plates and broth for 7 days.

4.1.7 Interpretation of Smears: Gram stain - Quantitate the presence of pus cells and organisms.

4.1.8 Interpretation of Cultures:

4.1.8.1 Examine the aerobic culture plates after 24- and 48-hours incubation and the anaerobic plates after 48 hours incubation.

4.1.8.2 Count the number and types of organisms.

4.1.8.2.1 If there are <3 types in total of organisms isolated, work up significant isolates as follows:

4.1.8.2.1.1 Workup any number of Probable Pathogens

4.1.8.2.1.2 Workup Possible Pathogens if pure growth OR moderate to heavy and obviously predominant growth.

4.1.8.2.1.3 Do not workup Skin Flora.

4.1.8.2.2 If there are >3 types in total of organisms isolated, work up significant isolates as follows:

4.1.8.2.2.1 Workup any number of Probable Pathogens

4.1.8.2.2.2 Do not work up other organisms.

4.1.8.3 Examine the THIO daily for evidence of growth. If no growth on culture plates but evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, MAC, CHOC and BA (anaerobic) and incubate and process as above. Keep the THIO at room temperature for a total of 5 days before discarding.

4.1.8.4 All isolates are to be identified as appropriate.

4.2 **Susceptibility Testing:** Refer to Susceptibility Testing Manual.

4.3 **Reporting Results:**

4.3.1 Gram stain: Report with quantitation the presence of pus cells and organisms.

4.3.2 Culture:

4.3.2.1 Negative Report: "No growth after 48 hours incubation" OR "Commensal flora"

4.3.2.2 Quantitate all significant isolates; report with appropriate susceptibility results. If other organisms are also present, report as "Commensal flora" with quantitation.

5. MATERIAL AND EQUIPMENT:

5.1 Routine culture media
5.2 Gram stain reagents
5.3 O2 & CO2 incubators
5.4 Microscan panels/ Vitek 2 system ID & AST cards
5.5 Anaerobic system (Jar & sachet)
5.6 Routine culture media

6. RESPONSIBILITIES:

6.1 The assigned technician/ technologist for microbiology lab.
6.2 The C. Pathology Specialist/ Consultant.

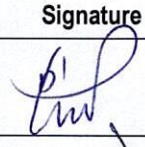
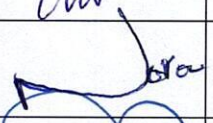
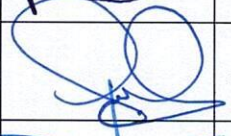
7. APPENDICES:

7.1 Organisms for workup.
7.2 Important information about tissue biopsies.

8. REFERENCES:

8.1 Procedure Manual, Toronto Medical laboratories / Mount Sinai Hospital department of microbiology.
8.2 H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 21. Edition, Vol 1 ASM Press, Washington, D.C.
8.3 H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 - 3.13.1.16. In Clinical Microbiology Procedures Handbook, 21° Edition, Vol 1 ASM Press, Washington, D.C.
8.4 H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 06, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 08, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025

Appendix 7.1

ORGANISMS FOR WORKUP

Probable Pathogens	Possible Pathogens	Commensal Skin flora
• Staphylococcus aureus • B-haemolytic streptococcus groups A, C, G. • B-haemolytic streptococcus group B (for neonates and postpartum patients only) • Streptococcus anginosus group(except tracheal swabs) • Pseudomonas aeruginosa • For chest tube drainage and tracheal swabs, include: Hemophilus influenzae. Streptococcus pneumoniae. • For sternal wounds, include: Coagulase-negative Staphylococcus	• B-haemolytic streptococcus group B (except for neonates and postpartum patients) • Viridians Streptococcus group • Enterococcus species • Aerobic gram-negative bacilli other than P. aeruginosa • Yeasts • Anaerobes	• Coagulase-negative Staphyl-ococcus (except sternal wound) • Micrococcus species • Corynebacterium species • Bacillus species not B. anthracis • Propionibacterium species • Non-pathogenic Neisseria species

Appendix 7.2

IMPORTANT INFORMATION ABOUT TISSUE BIOPSIES

Skin biopsies culture:

1. A variety of organisms may be associated with skin lesions and thus any growth of organisms other than skin commensal should be considered significant.
2. Reporting Results:
   - Gram stain: Report with quantitation the presence of pus cells and organisms.
   - Culture:
     - Negative Report: "No growth" or "Commensal flora"
     - Positive Report: Quantitate all significant isolates with appropriate sensitivities.
     - If commensal flora is also present, report with quantitation.

Placenta Swab/Tissue and Products of Conception Culture:

1. Although any organism may cause infection of the placenta, the most common organisms associated with this syndrome include S. aureus, beta hemolytic streptococci, Listeria monocytogenes and E. coli.
2. Reporting Results:
   - Gram stain: Report with quantitation presence of pus cells and organisms.
   - Culture:
     - Negative Report: "No significant growth" or "No growth".
     - Positive Report: "Neisseria gonorrhoeae isolated", (do not Quantitate),
     - Report all other significant isolates with appropriate sensitivity results.
     - Inform infection control of all Gonococcus (GC) isolates.

Endometrial Biopsies and Curetting culture:

1. The microbiologic diagnosis of endometritis is difficult. Anaerobes play an important role in this infection. However, most cases of endometritis follow childbirth, and it has been demonstrated that in the postpartum period, whether or not there is endometrial infection, significant numbers of anaerobes and other organisms from the cervical and vaginal flora may be found in the uterine cavity. Curetting may also be submitted specifically for Mycobacterium tuberculosis (TB) examination.
2. Reporting Results:
   - Gram stain: Report with quantitation the presence of the pus cells and organisms.
   - Culture:
     - Negative Report: "No significant growth" or "No growth." "No Neisseria gonorrhoeae isolated."
     - If CHOC plate is overgrown by swarming Proteus or yeast, report only as "Unable to rule out Neisseria gonorrhoeae due to bacterial/yeast overgrowth."
     - Positive Report: *"Neisseria gonorrhoeae isolated (do not Quantitate)* Quantitate and report all other significant isolates with appropriate sensitivity results.
     - Inform ordering Physician & infection control of all positive GC isolates.