Tissue Culture

Purpose

This procedure provides instruction for Tissue Cultures for the Microbiology Laboratory.

Principal and Clinical Significance

Organisms that reside on the skin and mucous membranes as well as organisms in the environment can cause infections if they enter normally sterile tissue through breaks in the skin or normally intact mucous membranes. Because virulence factors are not always necessary, virtually any species can be involved. Tissue specimens are obtained during surgical procedures at significant risk and expense to the patient. Therefore, it is important that pathology review these tissues. Tissues obtained at autopsy are valuable in determining the cause of death.

Policy Statements

This procedure applies to Microbiologists who perform culture set-up and plate reading.

Test Code

TISC

Materials

	Reagents	Supplies	Equipment	Media
	• Gram Stain reagents	• Sterile Forceps • Sterile Scalpel • Glass Slide (GMST) • Petri dish, Sterile • Sterile disposable pipette • Tissue grinding kit or sterile stomacher bag • Inoculating loop	• Ambient air incubator • Anaerobic jar • Anoxomat • CO_2 incubator • Incinerator • Microscope • Stomacher	Refer to the Sunquest specimen label for media information. • Chocolate agar (CHOC) • Sheep Blood agar (SB) • CNA agar (CNA) • MacConkey agar (MAC) • Normal Saline, 1 mL (SLNE) • Thioglycolate (THIO)

Specimen

A. Acceptable specimens
- Tissue

B. SDES codes/Specimen type
- State specific site of specimen.
- The code Sunquest code TIS cannot be used as a SDES code. It is considered a source code and will error in Cerner PowerChart.
- If the tissue is from an autopsy, add the code AUT after the site code.

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C. Special instructions
1. All surgery tissues need to be reviewed and documented by pathology before processing in microbiology, if surgery did not also submit to Pathology.
2. Process immediately. Do not allow drying out.
3. Aliquot the appropriate amount of specimen for other requests such as MC 1.02 AFB Culture or MC 1.17 Fungal culture.
4. **If there is a small amount** of specimen, call the physician to prioritize the tests.
- **If there is extra** tissue, save a sterile aliquot in a small amount of THIO or SLNE at 4ºC for 1 week in case further studies may be requested.

For additional information, refer to Lab Test Directory – Tissue culture and gram stain

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***.**

- *Biohazard Containment*
- *Biohazardous Spills*
- *Safety in the Microbiology Laboratory*

Procedure

A. Inoculation

1. Allow all media to come to room temperature before inoculation
2. Label all plates, tubes and slides properly with the patient's name, accession number and date.
3. 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.
4. Always inoculate the culture media first before preparing the slide when using the same pipette.

B. Specimen processing

PRION PRECAUTION: FOR BRAIN and SPINAL CORD TISSUE: Cover hood work surface with a disposable plastic backed pad, use disposable items for processing and discard all items into red trash for incineration. Immediately clean hood surface with 1:10 dilution of bleach. **Rinse well with water.**

1. **PREFERRED METHOD—**Stomacher;

DO NOT USE FOR HARD TISSUES that would puncture the stomacher bag.

a. Place tissue in sterile petri dish and cut into small portions using sterile forceps and scissors. Aliquot for additional testing (Fungal, AFB, etc.)
b. Using a sterile swab or forceps, transfer the tissue into a sterile Stomacher bag and add 1.0 ml of SLNE to moisten the tissue for homogenizing.
c. Insert Stomacher bag between the door and paddles in the blender, allowing 4 cm of the bag to project above the top of the door.
d. Pull handle forward to firmly close the door and switch machine on for 1-2 min.
e. Switch machine off, hold bag, open door by lifting the handle, and remove the bag.
f. Remove the all of the saline eluted specimen by using a sterile pipette.
g. Place 1-2 drops directly on each plate, into the THIO and onto a slide. Also, place a piece of the tissue into the THIO.
h. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.

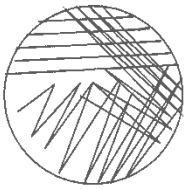
Note: Do not stomach or grind tissues for fungal culture. This will break up the fungal elements and prevent growth

2. **FOR BONE:** Tissue grinding method

a. Place tissue in sterile petri dish and cut into small portions using sterile forceps and scissors.
b. Using a sterile swab or forceps, transfer the tissue into the tube and add 1.0 ml of SLNE to moisten the tissue for grinding/homogenizing.
c. Using a circular motion, homogenize the specimen.
d. Remove all of the saline eluted specimen by using a sterile pipette.
e. Place 1-2 drops directly on each plate, into the THIO and onto a slide. Also, place a piece of the tissue into the THIO.
f. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.

3. Streak plates semi-quantitatively for primary isolation.

a. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
b. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
c. 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.
d. 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 3-4 times.



C. I**ncubation**

1. Incubate CHOC, SB, and CNA in 4-10% CO_2 at 35ºC
2. Incubate MAC and THIO in ambient air incubator at 35ºC.

D. Gram stain examination

1. Perform Gram stain and interpret.
2. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
3. Blot excess oil. Hold slide for one week. Hold autopsy slides for possible review in autopsy slide box.
4. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.

E. **Culture examination**: Read plates daily for 3 days.

Caution: For cultures of lymph nodes, work up slow growing organisms in BSC, since pathogens such as *Francisella, Brucella, and Mycobacterium* can be found in these specimens. See MCVI 3.60 Bioterrorism Protocol for LRN procedures for testing flowcharts of possible Bioterrorism organisms.

1. Day 1
   a. Examine aerobic plates and THIO.
   b. Plated media
      1. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase.
      2. Correlate colony types with the direct Gram stain.
      3. 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.
      4. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
      5. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
      6. Subculture organisms that are not well isolated to appropriate media for further work-up.
      7. Re-incubate primary plates and subcultures for an additional day.
      8. Report preliminary results.
      9. Culture plates that are no growth after 1 day should be taped closed and labeled as 'NG1-work up in hood'. These cultures may be a slow growing highly infectious organism and should be worked up in the BSC for our own safety. Please refer to the MCVI 3.60 Bioterrorism Protocol for more specific information.
   c. THIO broth
      1. Visually inspect THIO.
      2. If growth is observed, perform gram stain on THIO.
      3. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the smear correlates with the growth on the plates. Discard after 2 days.
      4. Subculture THIO's that are turbid when plates are negative for growth and Gram stain is no organisms seen.
      5. If there appears to be additional organisms in the THIO that are not on the plates, determine if Anaerobic Culture has been ordered.
         - If Anaerobic Culture has **not** been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
         - If Anaerobic Culture has been ordered, subculture to appropriate aerobic media. Identify appropriate organisms.

- If organism in THIO appears to be an anaerobe, hold THIO for 5 days. After 4-5 days, confirm isolation of organism in Anaerobic Culture before finalizing culture.

2. Day 2
   a. Examine primary plates from the previous day for additional microorganisms.
   b. Read and record identification tests and susceptibilities from the previous day.
   c. Set up additional tests as needed.
   d. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section 'c' above for further instructions.
   e. Ensure THIO with growth was gram stained for 2 consecutive days.
   f. Send updated report.
   g. Call MRSA results to the patient's caregiver, if not previously positive.
3. Additional Days
   a. Complete identification and susceptibility testing procedures until all significant isolates are finished.
   b. If there is no growth on the plates, discard after 3 days. Culture is held open while THIO continues to incubate.
   c. Hold the THIO for 5 days. If no growth in THIO, final the report as "No Growth, 5 days".
   d. Send updated report and finalize
   e. 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.
   h. Save a representative primary plate for anaerobes in an anaerobic jar or bag, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.

Limitations

Slow-growing *Mycobacterium* sp. or *Nocardia* sp. that may cause abscesses will **not** be recovered in routine bacterial cultures even if present, since extended incubation periods or special media are necessary for isolation. Cultures for these organisms should be specifically requested.

Critical/ Significant results

1. *Francisella* can be found in lymph node biopsy specimens and is extremely infectious. It is a tiny coccobacillus that grows slowly, is catalase positive and oxidase negative. Work in BSC if suspected. Do not use automated ID systems. Please refer to the MCVI 3.60 Bioterrorism Protocol for more specific information.

2. Notify physician of the isolation of ***S. pyogenes*** which can cause life threatening necrotizing fasciitis.

3. Critical Results are reported within 60 minutes to the provider. Refer to MCVI 4.0 Critical Results procedure for full list. Organisms to watch for include:
   - MRSA
   - ESBL or Carbapenemase producers
   - **Agents of Bioterrorism**--Bacillus anthracis, Brucella, Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis, or Yersinia pestis
   - Fungal or Yeast isolates:
     - Mucorales-all genera included in this Order, including those listed below
       - Mucor spp.
       - Rhizomucor spp.
       - Rhizopus sp.
       - Syncephalastrum spp.
       - Lichtheimia spp.
       - Cunninghamella spp.
     - Cryptococcus neoformans
     - Coccidioides immitis
     - Histoplasma capsulatum

- Blastomyces dermatitidis
- Sporothrix schenkii

- VRE
- Corynebacterium diphtheriae

Method Performance Specifications

1. If there are more isolates to report than there are available lines in Sunquest it will be necessary to create a continued report.
2. In Order Entry, order MMCC (Miscellaneous Culture Continued Report using the same date/time of collection.
3. Add "SEEC" to the original accession in line 9 and "RCON" to the new accession in line 1.
4. It will be necessary to free text the new and old accessions after the SEEC and RCON comments. Refer to MCVI 5.0 Micro Computer Training for complete details.

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+.

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

a. **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 5 days".

b. Positive cultures:

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to follow

Workups:
Wkup # 1
Med : SB
Desc : BH
Id : SAUR

Workup Components
GMS : STPH
SC : SB
SLC : POS
VMIC : 1

c. **If growth is only in the THIO,** report as:

Observations: 1. GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow. (**GNR-BO-FID**)

Workups:
Wkup # 10
Med : THIO
Desc : CLDY

Workup Components
SC : SB MAC
GMS : GMNR

d. **Gram stains**: Report Gram stain results by selecting the *Direct Exam* tab. Follow MC 2.1 Gram Stain procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI
2. 4+ WBC'S

e. Call MRSA results to patient's caregiver, if not previously called. Document date and time called in computer.

3+ METHICILLIN-RESISTANT STAPH AUREUS ***MDRO***
MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.
**Called to Linda S., RN L8 @ 1300 7/7/03

f. 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.
   - Re-final the culture when identifications and/or testing are complete.

g. 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 Mislabeled / Unlabeled Specimens and Correcting Patient Data for Sunquest report entry information.

References

1. Leber, Amy. Clinical Microbiology Procedures Handbook, 4th edition. 2016. Vol. 1-3. Section 3.13. American Society for Microbiology, Washington D.C., 20036.

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.

Appendices

WORKLABEL MEDIA-FORM DEFINITION

BATTERY: TISC

SPEC	MEDIA
0	SLNE, GRIN, CHOC, SB, CNA, MAC, THIO, GMST

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Pat Ackerman	1978	Initial Version
1.1	Pat Ackerman	04/1979	
1.2	Pat Ackerman	02/03/1992	
1.3	Pat Ackerman	08/11/2003	
1.4	Pat Ackerman	01/22/2006	
1.5	Pat Ackerman	01/11/2008	Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Revised MRSA reporting. Added procedure notes 1 and 2.
1.6	Jessica Craig / Becky Carlson	10/26/2010	Updated into online format
2	Becky Carlson	4/18/2015	Re-numbered from MC 430 for CMS load.
3	Susan DeMeyere	9/7/2017	Changed reporting to keep culture open while THIO is incubating. Added to tape closed and label plates with no growth as NG1-work up in hood.

	4	Susan DeMeyere	10/30/2018	Removed culturing for anaerobes on initial set up. Added instructions for THIO processing.
	5	Susan DeMeyere	10/9/2020	Removed use of Scant when growth only from THIO.
	6	Susan DeMeyere	10/1/2024	Added additional THIO instructions.