POLICY STATEMENT	The initial processing of clinical specimen for bacteriology is a multifaceted endeavor involving a number of decision-making steps, including the need for processing the specimen for gram stains, aerobic, anaerobic, fungal, and mycobacterial cultures. These issues will determine whether the specimen requires any pretreatment before inoculation. First one must consider the specimen type and its anatomic origin. The second step is the selection of primary isolation media to be used for each specimen type. The final step is the selection of incubation temperature and atmosphere.
PURPOSE	This procedure provides technical instruction for the performance of the specimen processing.
SCOPE	This procedure applies to testing personnel authorized to perform testing. This group includes, but is not limited to Laboratory Technologists as well as leads and supervisory personnel.
RESPONSIBILITY	All the above personnel are responsible for following the specimen processing procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
RELATED DOCUMENTS	MICR 6140 Ja Transfusion Reaction Blood Cultures MICR 6140 Jb Set Up Chart MICR 6140 Jc Media Chart MICR 6140 Jd Micro Specimen Processing MICR 6140 Je Creating an Account Non-Patient Testing MICR 6335 R Environmental Cultures MICR 6335 J Water Cultures MICR 6370 J BAL Quantitative Cultures MICR 6200 R Gram Stain

SPECIMEN HANDLING

Proper Specimen collection is critical to isolating the causative agent of infection. See the Laboratory Test Directory and Laboratory Service Manual located on the SAINT for specimen collection and transport information.

Specimen Aliquoting Requirements:

- Always utilize a fresh pipette any time material is aspirated.
- Never add a sample into an unlabeled container.
- Never mix sample types in one container.
- Never return an aliquot to the original container.
- The original container and the aliquot tube must be legibly initialed by the person performing the aliquoting.
- If a label is placed over an existing label, the new label must be initialed by the person performing the task.

MEDIA

- Enrichment Media: Nutrients have been added to enhance the growth of bacteria.
- Differential Media: The media contains carbohydrates, indicators and chemicals that will differentiate different types of bacteria.
- Selective Media: This media contains certain carbohydrates, indicators and chemicals that are inhibitory to certain groups of bacteria.
- For appropriate selection of media see MICR 6140 Jb Set Up Chart and MICR 6140 Jc Media Chart

Store at Media at 2ºC to 8ºC. DO NOT use the beyond the expiration date.

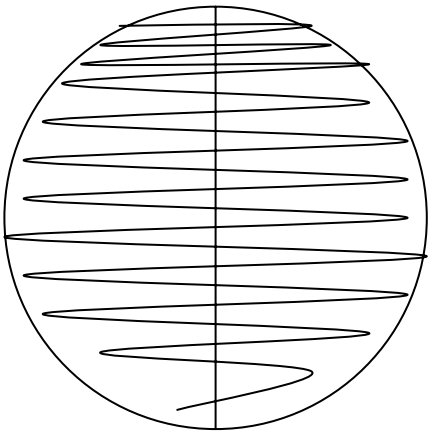
QUALITY CONTROL

Quality Control is performed as outlined in CLSI Approved Standard M22: Quality Control for Commercially Prepared Microbiology Culture Media

PROCEDURE

The streak plate method is a rapid and simple technique of mechanically diluting a relatively large concentration of microorganisms to a small, scattered population of cells. The goal is to obtain isolated colonies on a large part of the agar surface, so that desired species can then be brought into pure culture. Proper streaking of plates is an indispensable tool in microbiology. In most cases a closed inoculating loop is used for streaking plates.

- All specimens are to be plated in the level II biological safety cabinet.
- Labeling:
  - Test request specimens through computer and obtain accession number for the cultures. See MICR 6140 Jd Micro Specimen Processing.
  - Place the small aliquot computer label on plates and tubes being careful not to cover the name of the media or expiration date.
  - Place string number on plates.
- Urines cultures, do not streak in quadrants. Instead use the appropriate calibrated loop (Inoculate with white - 0.001 ml or blue - 0.01 ml). Streak plate according to this pattern



- Other Specimen types:
  - If swabs are received, use one swab for the culture and the other swab for the gram stain. Discard the swab that was used for the gram stain. If only one swab is received, inoculate media first, and then make the smear. If a fluid or tissue specimen is received on a swab enter the comment:
    SWAB = Specimens received on swabs are inferior to samples of tissue or fluid.
  - If a sterile body fluid is received and the volume is >1cc and clot is not present, centrifuge for 15 min at 2500 RPM's using the sediment for culture and the cytocentrifuge is used to make the direct slide for staining. If clot is present, break up the clot and set directly. **For ALL joint fluids**, also inoculate a pediatric blood bottle with up to 4 ml of fluid. Add 2.25ml BHI with Fildes to the bottle and load into the BacT/Alert.
  - If a bronch wash is received and the volume is less than 10 ml set up the specimen directly. However, if the volume is greater than 10 ml, remove 2 ml, centrifuge for 15 min at 2500 RPM's and work with the sediment for the culture.
  - If a BAL is received, set up the quantitative culture. See MICR 6370 J BAL Quantitative Cultures.
  - If a tissue is received, mince tissue into 1 mm. cubes and emulsify with tissue grinder in sterile saline.
  - If the specimen cannot be ground such as a graft, bone or IUD, add the specimen to Thioglycollate Medium and incubate overnight. Vortex the thio and immediately inoculate the appropriate solid media and incubate.
  - If pathology calls and says that they have a frozen section, take a sterile petri dish and forceps with you to the frozen section lab (the second door on the left after cytology and before the double doors). The pathologist will give you a small piece of tissue to grind like any other tissue. There may not be paper work. Ask for the patient's demographics and tests requested. Enter the orders in Meditech and process appropriately.

- For all specimen types, inoculate media according to set up chart using a sterile swab or pipette. See *MICR 6140 Jb Set Up Chart*
- Inoculate the upper 1/3 of the solid media starting with the most nutritious media and least selective media (e.g. chocolate agar)
- Method for streaking plates
  - Position the plate so that the spot of inoculum is nearest the hand not holding the loop (the opposite hand).
  - Use a sterile disposable loop, streak with gentle pressure. Use the method shown below to avoid tearing the agar.

http://web.indstate.edu/thcme/micro/basic.html

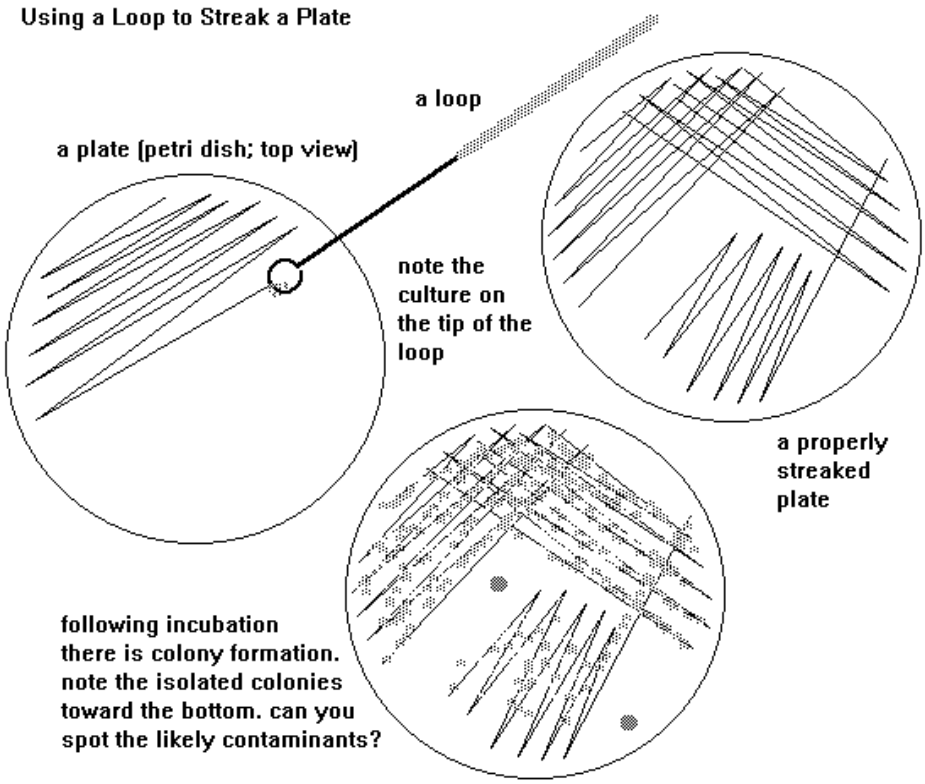
  - Move the loop back and forth across the spot and then gradually continue toward the center of the plate as you sweep back and forth. Use a very gentle and even pressure.

http://web.indstate.edu/thcme/micro/basic.html

  - When creating the first quadrant, do not worry about keeping each pass across the plate separate from previous ones.
  - When about 30% of the plate has been covered by the first streaking quadrant, rotate the plate and repeat the above procedure with the same loop

for the second quadrant, but this time pick up some inoculum by crossing into the first quadrant 2-3 times and then not passing into it again.

- Repeat the same procedure for the third quadrant. After streaking the plate, discard the loop. Use a new loop for each plate to avoid carry over.



http://www.mansfield.ohio-state.edu/~sabedon/biol4035.htm

- Catheter tip cultures:
  - Using sterile forceps transfer the catheter segment onto the surface of a BAP. If Tip is longer than 2 inches, using a sterile scalpel, cut off the tip to culture.
  - With light pressure from the forceps, roll the catheter back and forth across the plate at least four (4) times.
  - Return the catheter tip to the specimen container. Save with daily Specimens
  - Urinary Foley Cath Tips and Tips received in saline or transport media are unacceptable specimens for culture.

- When multiple types of cultures are requested, bacterial cultures should be set up first.
- If fungus cultures are requested, inoculate Sabouraud Dextrose agar and Mycosel agar
- For Mycobacterial cultures put 5-10 ml of the specimen in a 50ml conical tube to be processed. See the procedure for Mycobacterial cultures.
- Incubate plates with the lid down in the designated 35°C CO_2 incubator for 18-24 hours.
- Incubate fungal tubes in the 30°C incubator.
- Place anaerobic cultures in the appropriate environment by using the appropriate Gas Pak and place in the 35°C non CO_2 incubator.

  NOTE: Place the inoculated pates inside the resalable pouch. The Campy pouch requires at least 2 plates and will hold up to 4 plates. The CO_2 and anaerobe=e pouch hold 1 to 4 plates. For optimal growth using the campy pouch, place a gauze moistened with 5 ml of sterile water in the pouch.
- Archive and save all specimens in the refrigerator for 1 week. Save all sterile body fluids and other irretrievable specimens for 4 weeks.

<u>Rejection of Specimens</u>

1. Cancel the specimen and enter the appreciate comment.
2. Notify the caregiver:
   - Inpatients and ED: Notify Team Leader via SmartWeb or the unit by phone.
   - Outpatients: Give the information to the phone bank. They will notify the physician office.
   - Document the notification in Meditech.

REFERENCES

Garcia, L. Clinical Microbiology Procedure Handbook. 3nd Edition, Volume 1, Section 3, American Society for Microbiology, Washington, D.C

Bailey and Scott's Diagnostic Microbiology 11th edition, Mosby, Inc

BBL Quality Control and Product Information Manual for Plated and Tubed Media Becton Dickinson and Company, June 2009

CLSI Approved Standard M22: Quality Control for Commercially Prepared Microbiology Culture Media

CREATING AN ACCOUNT FOR NON PATIENT TESTING

From the SPECIMEN DESKTOP select “Enter/Edit Req.” on right hand side bar
Enter the following information in the given fields.

- **Patient:**
  - Autopsy specimens = Autopsy, A(year)-(number) e.g. A14-06
  - Chemistry water = Corelab, QC
  - Histology water = Histolab, QC
  - Infection Control specimens = Infection Control, QC
  - Microbiology specimens = Micro, QC
  - Pharmacy specimens = Pharmacy, QC
  - Proficiency samples = SURVEY(year), (survey organization)_(ID on vial)
    Example: SURVEY2010, CAP CHM-11
- Enter a New Account
- Facility – LAB CENSUS
- Registration Screen – REFS
- Age – 99
- Sex – U
- Financial Class – U
- **Client:**
  - Autopsy specimens = LAB
  - Chemistry water = LAB
  - Histology water = LAB
  - Infection Control specimens = INF
  - Microbiology specimens = LAB
  - Pharmacy specimens = PHR
  - Proficiency samples = LAB
- Attending – NONE except for Autopsy’s enter the pathologist’s name
- Select SAVE
- ADM Priority – OTHER (OTH)
- **Location:**
  - Autopsy specimens = LABML
  - Chemistry water = LABML
  - Histology water = LABML
  - Infection Control specimens = INF
  - Microbiology specimens = LABML
  - Pharmacy specimens = PHR
  - Proficiency samples = LABML
- Select SAVE
- Admission Form – N
- Plate – N
- Select OK
- Proceed with test request.