

CULTURE COLLECTION

I. GENERAL:

The Mycology culture collection is comprised of fungi and aerobic actinomycetes that have been preserved for future reference and Quality Control. Unusual or significant isolates should be placed in the culture collection. All isolates which have had drug susceptibilities determined are also placed in the culture collection. Refer isolates for the collection to the senior technologist.

Cultures can be preserved, maintaining viability, using four different techniques; water culture, freezing at -70°C , mineral oil overlay, and by freeze drying. All four techniques should be employed, when appropriate, to insure the viability of the isolate during prolonged storage. Routinely, only -70°C storage is used.

When retrieving isolates, subcultures can be made easily from the water culture or from mineral oil overlay slants. When a frozen slant is used for subculture, it must be replaced with a new slant if the culture has thawed. Do not refreeze a slant that has been frozen and then thawed. Frozen isolates should not be out of the freezer for more than 5 minutes.

II. PROCEDURE FOR CULTURE COLLECTION ADDITIONS:

- A. Confirm the purity and identification of the isolate by sub-culturing onto PDA and performing the necessary tests and examinations.
- B. Assign the isolate an accession number and label the PDA sub-culture plate with that number.
- C. Prepare a slide culture using PDA and 2 subcultures on PDA slants (reusable glass screw cap tubes).
- D. Prepare a permanent LP mount from the slide culture and label with the accession number and file in the collection slide boxes.
- E. When the subcultures have sporulated, place the two labeled slants at -70°C in the Revco freezer.
- F. Fill out a culture collection data sheet. If the isolate was sent to UTMB, include the name and address of the sender, the collection data and additional reference numbers from other institutions.
- G. Put the data sheet in the culture collection file in numerical order.
- H. On an index card, type in the name of the isolate and the culture collection accession number. File the index card in the culture collection file box in alphabetical order.
- I. When a subculture from the culture collection is given or sent to someone, record the name and address of the recipient, the date sent and the initials of the person who prepared and mailed the subculture.

CULTURE COLLECTION

III. FREEZING TECHNIQUE:

A. Procedure for Preparing Cultures for Storage:

1. An actively sporulating culture on a PDA slant is labeled with the accession number.
2. Tighten the screw cap. Reusable glass tubes must be used since disposable glass tubes may break in the freezer.
3. Plate the slant in the appropriate rack in the Revco freezer which is maintained at -70°C.
4. Dangerous fungi such as *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Xylohypha bantiana* must be packed in crush-proof containers before freezing, and labelled on the exterior of the container.

B. Procedure for Preparing Subcultures:

1. Remove the frozen PDA slant from the freezer.
2. Open the tube and remove a small amount of the colony from the frozen agar surface with a long handle inoculating needle or pipette. Return the slant to the freezer unless it has begun to thaw (more than 5 minutes). If the slant thaws, make a fresh subculture to replace it in the freezer.
3. Place the inoculum on a PDA plate labelled with the accession number. Streak out the inoculum with a loop or needle.
4. Incubate the plate at 30°C until growth appears.
5. Prepare an LP mount and confirm the identity of the isolate.
6. Prepare subcultures as needed. If subcultures are sent to other institutions or persons, record the information on the culture collection data sheet.

C. Procedure for Preparing Subcultures from Mineral Oil Overlay Cultures:

1. Flame the neck and cap of the slant.
2. Unscrew the cap and re flame the neck of the tube.
3. Remove a small amount of the colony through the oil overlay using a sterile long handle inoculating needle or pipette. Avoid any string-like growth that reaches into the oil. Replace the screw cap.
4. Drain as much mineral oil as possible from the inoculum.
5. Place the inoculum into a tube of Sab's broth labelled with the accession number.
6. Incubate the tube at 30°C until growth appears. Subculture to a labeled PDA slant. Return the oil overlay slant to the culture collection rack.
7. Prepare an LP mount and confirm the identity of the isolate.
8. Prepare subcultures as needed. If subcultures are sent to other persons or institutions, record the information on the culture collection data sheet.

IV. WATER CULTURE TECHNIQUE:

A. Procedure for Preparing Cultures for Storage:

1. Aseptically add 2 ml of sterile distilled water to an actively sporulating culture on a PDA slant.
2. Dislodge conidia with a sterile long handle inoculating needle avoiding digging into the agar.

CULTURE COLLECTION

IV. **WATER CULTURE TECHNIQUE:** (cont'd)

3. With a sterile capillary pipette, remove the suspension and transfer it to a sterile 1 dram vial labelled with the accession number. Yeast colonies can be directly transferred with an inoculating loop to a sterile vial containing 2-3 ml of sterile distilled water.
4. Screw the cap down tightly and store the vial at room temperature in the appropriate culture collection box.
5. Additional sterile water may be added at any time to prevent dehydration.

B. Procedure for Preparing Subcultures:

1. Flame the neck and cap of the vial.
2. Shake the vial to resuspend the fungus.
3. Unscrew the cap and re flame the lip of the vial.
4. Aseptically transfer 0.2 - 0.5 ml of the suspension to a PDA plate labelled with the accession number.
5. Tighten the cap and return the vial to storage.
6. Incubate the PDA plate at 30°C until growth appears.
7. Prepare subcultures as needed. If subcultures are sent to other persons or institutions, record the information on the culture collection data sheet.

V. **PROCEDURE FOR NON-VIABLE SUBCULTURES:**

A. Sabouraud dextrose broth subculture.

1. Inoculate the apparently non-viable isolate into 2-3 ml of sterile Sabouraud dextrose broth labelled with the accession number.
2. Incubate the tube at 30°C and examine for the presence of new growth.
3. If new growth appears, transfer to a PDA slant labelled with the accession number.
4. If no growth is present after 3-4 weeks, the culture is non-viable.

B. Check the culture collection data sheet to see how many techniques were used to preserve the culture. Then try to subculture the isolate from each stored unit.

C. If subculturing from each unit fails to yield a viable isolate, check the data sheet to see if the isolate was sent to another person or institution. Write and request that a subculture be sent from their collection.

D. After all attempts to subculture or request the isolate have failed, write on the data sheet in red ink that the culture is non-viable, the date and initial. Return the data sheet to the file.

VI. **REFERENCES:**

McGinnis, MR: Laboratory Handbook of Medical Mycology, New York, Academic Press, 1980.

CULTURE COLLECTION
CULTURE COLLECTION DATA

Identification

Accession Number

Sender: _____

Date Received

Identification by:

Purity checked:

Storage:

Additional reference numbers:

Collection data:

Notes: