

Techniques for the Microscopic Examination of Fungal Culture

Identification of Fungi

Macroscopic Examination of Fungi:

- texture
- color in the plate

Microscopic Examination of Fungi:

- Lacto Phenol Cotton Blue Teased Mount (LPCB-TM)
- Double sided Sticky Scotch Tape
- Slide culture technique.

Lacto Phenol Cotton Blue Teased Mount (LPCB-TM)

Materials used:

Slide, cover slip, fungal colony, LPCB

Procedure:

1. Place a drop of LPCB on the slide.
2. Using a sterile iron needle, transfer a tiny piece of the colony into the LPCB on the slide.
3. Tease the colony into very tiny pieces using iron needles.
4. Cover the preparation with a cover slip.
5. Examine the wet preparation under the x40 objective

Lacto Phenol Cotton Blue Teased Mount (LPCB-TM)

Disadvantage:

- The intact morphology will not be seen

Advantages:

- It is the most widely used method for staining and observing fungi because it's easy and fast.
- This stain is useful for making permanent mount of the fungus which is in culture

Double Sided Sticky Scotch Tape

Materials used:

Slide, cover slip, fungal colony , LPCB, Double sided scotch tape, forceps

Procedure:

1. Stick one side of the scotch tape on the cover slip
2. Place the cover slip with the other sticky surface on the fungal colony, then press the cover slip onto the colony
3. Place a drop of LPCB on a slide
4. Using a forceps pick up the cover slip and transfer it onto the LPCB on the slide [The side onto which the colony is stuck should be in the LPCB]
5. Examine microscopically using the x40 objective

Double Sided Sticky Scotch Tape

Disadvantages:

- Only the superficial structures of the fungi tend to stick to the tape.
- This technique is rarely used because of the technical inconvenience.

Advantage:

- The fungal morphology appear intact compared to LPCB-TM.

Scotch



PERMANENT

Double Sided Tape

1/2"

**Permanent
Double Sided Tape**

1/2 IN x 130 IN
(3.61 YD)
12,7 mm x 3,3 m



Scotch

3M



Slide Culture Technique

- This technique is the best technique for the microscopic examination of mold cultures.
- But,, it takes time, some fungi take months to sporulate.
- The time and the nature of the block of agar are determinant factors.
- This technique allows the intact morphology of the fungus to be seen under the microscope.

Slide Culture Technique

Materials used:

Petri dish, slide, cover slip, filter paper, V-shaped glass rod, PDA, fungal colony, needle, forceps.

Procedure:

1. In an empty glass Petri-dish put : filter paper (7cm diameter), V-shaped glass rod, glass slide, and two cover slips.
2. Sterilize the set above by autoclaving at 121°C for 15 minutes.

Slide Culture Technique

3. Cut a PDA plate into small squares (approximately 1cm), then take one block and place it in the middle of the slide.
4. Using sterile needles inoculate the four sides of the PDA block with the fungal colony.
5. Using a forceps place a cover slip on top of the inoculated PDA block .
6. Wet the filter with some sterile distilled water to prevent drying of the agar block and incubate the set at 30 °C.

After 48 hrs of incubation growth and sporulation may appear.

7. Gently take the cover slip on the agar (block) and place it on slid with LPCB then examine under x40.

8. If the fungus is still underdeveloped , add a fresh cover slip on the agar block and continue incubation .