TECHNIQUES FOR THE MICROSCOPIC EXAMINATION OF FUNGAL CULTURES

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Identification of Fungi

1-Macroscopic Examination of Fungi :

1. **Texture**
2. **Color**

2-Microscopic Examination of Fungi :

1. **Lacto Phenol Cotton Blue Teased Mount (LPCB-TM)**
2. **-Double sided Sticky Scotch Tape**
3. **-Slide culture**

LACTO PHENOL COTTON BLUE TEASED MOUNT

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

***Disadvantage:**

The intact morphology will not **be seen**

***Advantage:**

Easy and fast

DOUBLE SIDED STICKY SCOTCH TAPE

This technique keeps the reproductive structures of the fungus intact as compared to LPCB-TM.

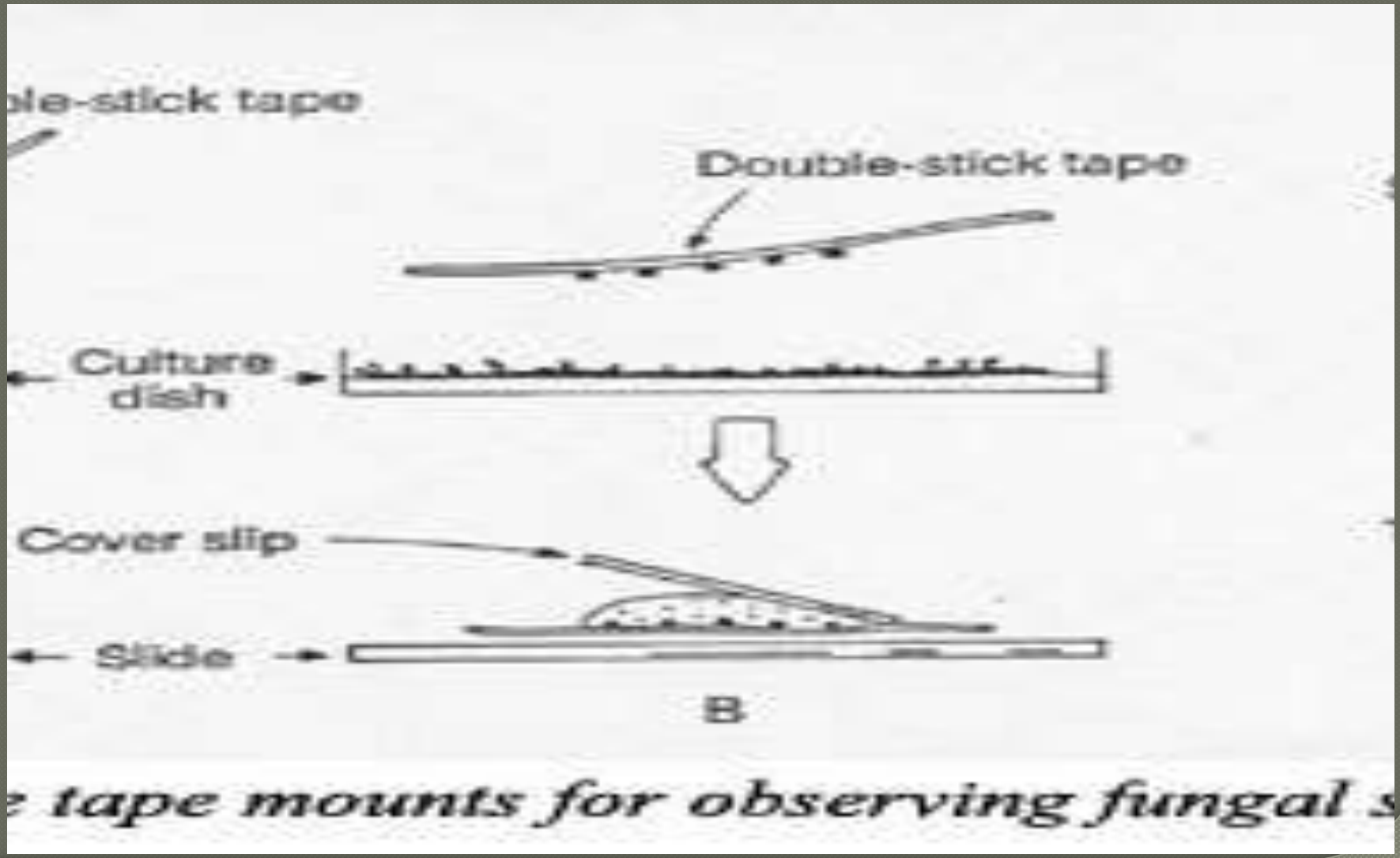
DOUBLE SIDED STICKY SCOTCH TAPE

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. Press the cover slip onto the colony.
3- place a drop of LPCB on a labeled 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



e tape mounts for observing fungal s

DOUBLE SIDED STICKY SCOTCH TAPE

Disadvantage:

1-Only the superficial structures of the fungi tend to stick to the tape.

2- The technique is troublesome(difficult) and awkward(uncomfortable) in inexperienced hands.

*Advantage:

The fungal morphology appear intact.

SLIDE CULTURE TECHNIQUE

- This technique allows the intact morphology of the fungus to be seen under the microscope.

- Slide culture technique is the best technique for the microscopic examination of mold cultures. However it takes time … some fungi take months to sporulate, and even after months, only sterile hyphae can be seen. Time and the nature of the block of agar are determinant factors.

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 : a 7cm. Diameter filter paper, a V-shaped glass rod , a glass slide, and two cover slips [see drawing]. Sterilize the set above by autoclaving at 1210C/15 minutes.

Slide culture technique …

Procedure:

2- Cut a PDA plate into small squares of approximately 1 cm. square each. Take one block of the cut PDA medium and place it in the middle of the slide.

3- Using sterile needles inoculate the four sides of the PDA block with the fungal colony under investigation.

4- Using a forceps place a cover slip on top of the inoculated PDA block .

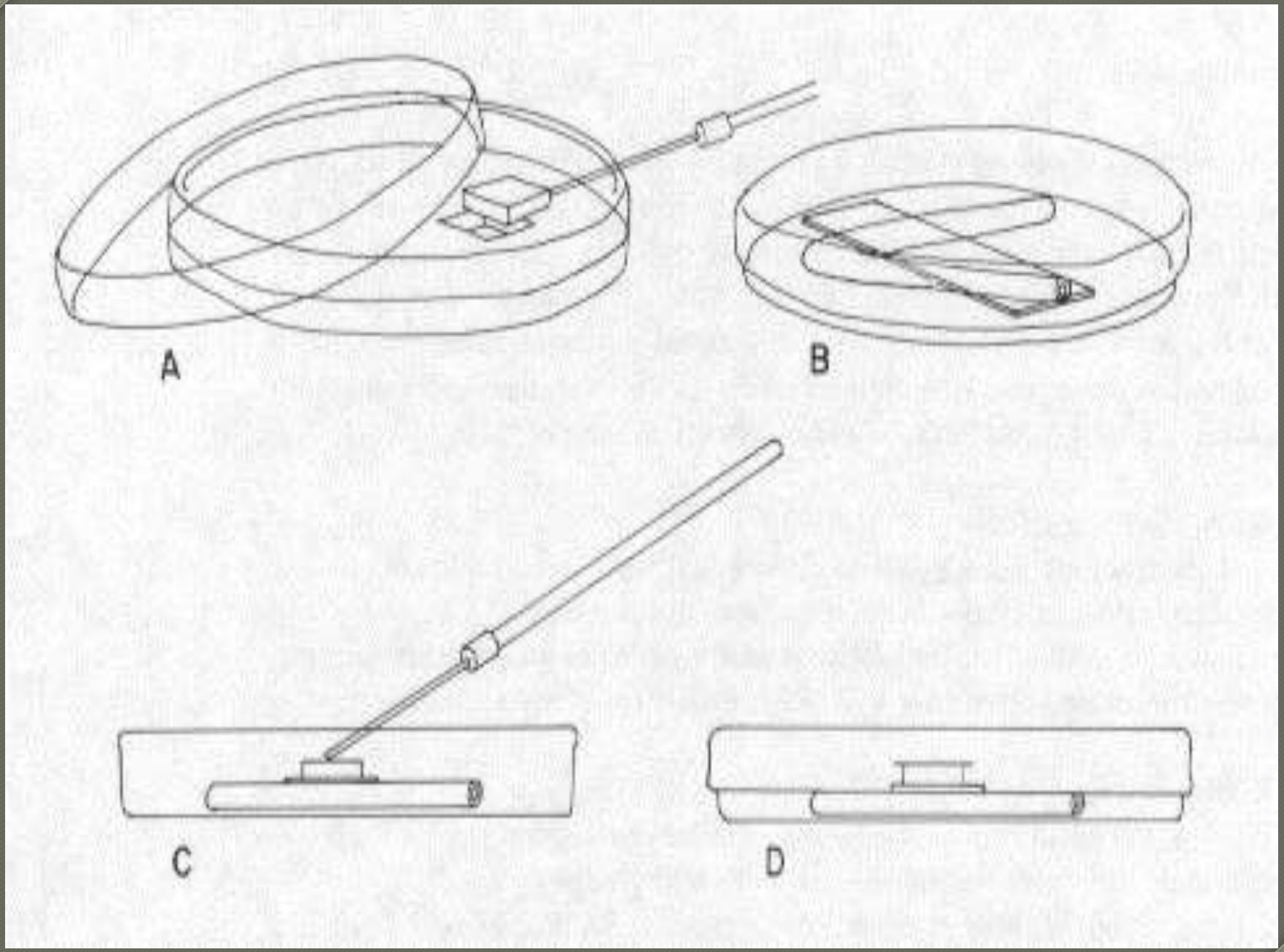
5. Wet the filter with some sterile distilled water to prevent drying of the agar block and incubate the set at 28 0C.

Slide culture technique …

5- Wet the filter with some sterile distilled water to prevent drying of the agar block and incubate the set at 28 0C. Inspect for growth and sporulation after 48 hours of incubation

6- Gently lift the cover slip of the agar and place it on slid with LPCB. Examine under x10 & x40.if the fungus is still underdeveloped , add a fresh cover slip on the agar block and continue incubation .

7- Also, we can discard the agar block , put 1 drop of LPCB on it and cove it with the cover slip and examine x10 & x40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