Lacto-Phenol Cotton Blue Preparations

1. Principle and Clinical Significance

When a mold is isolated on primary media, microscopic examination with lactophenol cotton blue stain (LPCB) can be performed to observe the manner of sporulation and to provide information for a possible organism identification. Lactophenol cotton blue is used both as a mounting fluid and a stain. Lactic acid aids in preserving the fungal structures, phenol acts as a killing agent, and cotton blue imparts color to the structures. Cotton blue (China blue) stains chitin, the primary component of fungal cell walls, and cellulose.

The tease mount method is the time honored method for preparation of LPCB preparations. Its major drawback is the difficulty in preserving intact reproductive structures that are essential for correct genus and species identification.

The cellophane tape method is a rapid way of studying microscopic morphology and successfully retains the original position of the characteristic fungal structures. However, cellophane tape may be cumbersome to work with, and the mounts cannot be saved indefinitely.

Polyvinyl alcohol (PVA) can be used as a substitute for scotch tape in the lactophenol cotton blue mount. PVA provides a sticky surface for the fungal elements to adhere to. PVA mounts maintain the original position of the characteristic fungal structures better than scotch tape mounts. The mounts can be sealed with nail polish and saved indefinitely.

II. Specimen

A. Actively growing mold colony
B. Coverslip from a slide culture, see **"Slide Culture"** procedure for slide culture set-up.

III. Materials and Reagents

A. Lactophenol cotton blue, store at room temperature until the stated expiration date. Label LPCB box with chemical sticker Health = 3, Flammability = 2, Reactivity = 0.
B. Cellophane tape
C. Clean glass slides
D. Pencil
E. Teasing needles
F. Coverslips
G. Bunsen burner
H. Coplin jar with alcohol and sand
I. Microscope with 10X and 40X objectives
J. Distilled water
K. Clear nail polish
L. Sterile plastic loops
M. 8% PVA (Wear gloves, mask, and safety glasses when preparing 8% PVA.)
    1. Dissolve 8 g of PVA (Sigma) in 100 mL of distilled water.
    2. Filter sterilize the PVA with a 500 mL 0.20 μ filter.

3. Store in 0.5 mL aliquots in microcentrifuge tubes at room temperature.
4. Use a new tube every week.
5. The PVA solution can be stored indefinitely as long as no contamination is visible.
6. If contamination is evident, discard the tube, and obtain another one.

IV. Safety

A. All of the LPCB preparations are done in the biological safety cabinet.
B. The handling of all cultures containing filamentous fungi must be performed in the biological safety cabinet.

V. Quality Control

A. Examine the LPCB for staining effectiveness on each day of use.
B. Perform a LPCB mount on a mold isolate according to the procedure below (VI.A.).
C. Observe the LPCB mount for characteristic staining.
    1. Hyphae absorb LPCB and demonstrate deep blue hyphae walls and septa.
    2. The interior of the hyphae stains pale blue.
D. Record the results.
E. Notify the supervisor if the staining reaction is not appropriate.

VI. Procedure

A. PVA - LPCB mount (preferred method)
    1. Remove one microcentrifuge tube of 8% PVA from storage rack.
        a. Label the tube with the date opened.
        b. The tube outdates 1 week from the date opened.
    2. Use a sterile loop to spread a thin layer of PVA on a 22 x 22 glass coverslip.
    3. Place the coverslip PVA side up on a paper towel.
        a. Allow the PVA to dry for 1 min.
        b. The PVA should be tacky.
    4. Label a glass slide with the culture number or the patient's last name. Place a drop of LPCB in the middle of the slide.
    5. Using sterile forceps, pick up the coverslip.
    6. Press the coverslip, PVA side down, on top of the mold colony.
    7. Lift the coverslip, and place the PVA side down on the LPCB.
    8. Examine the slide for representative fungal structures with the 10X and 40X objectives.
    9. The slide may be permanently saved by using nail polish to seal the coverslip edges.
B. Cellophane tape method
    1. Place a drop of LPCB on an appropriately labeled slide.
    2. Cut a 1" piece of cellophane tape.
    3. Holding the ends of the tape, press the sticky side very firmly to the surface of the fungal colony approximately 4-5 mm in from the periphery. Pull the tape away gently. Aerial mycelia will adhere to the tape.
    4. Turn the tape over so the non-sticky surface is in the drop of LPCB.
    5. Add another drop of LPCB, and coverslip.

6. Examine under 10X and 40X magnification.

C. Tease mount technique (perform tease preparations in the biological safety cabinet)
   1. Place one drop of LPCB on an appropriately labeled clean glass slide.
   2. With a flamed bent needle, remove a piece of the colony, and place it in the drop of LPCB.
   3. With two flamed dissecting needles, gently tease apart the mycelial mass.
   4. Coverslip, and examine under 10X and 40X power.
   5. Insert the dissecting and bent needles several times in the alcohol and sand mixture before flaming to eliminate spores.

D. Slide culture coverslips
   1. Place one drop of LPCB on an appropriately labeled glass slide.
   2. Remove a coverslip from slide culture, and place it on the slide.
   3. Add another drop of LPCB, and coverslip.
   4. Examine under 10X and 40X magnification for typical reproductive structures.

VII. Interpretation

A. Describe representative hyphal structures with characteristic sporulation in the computer.
B. Use the available mycology references to determine the identification of the mold.
C. Notify Rounds of the results to determine whether the mold will be reported or regarded as a contaminant (see **"Culture Evaluation"** procedure).
D. If an identification can not be made, it may be necessary to set up a slide culture. The isolate should be brought up on rounds to determine whether a slide culture is necessary.
E. If the mount is from a slide culture, another slide should be stained after several days to look for characteristic fungal structures.

VIII. Result Reporting

A. If the isolate can be identified based on the LPCB mount, report the genus and species of the isolate.

IX. Procedure Notes

A. PVA procedure
   1. Always examine the PVA tube for gross fungal contamination. Discard any PVA that is visually contaminated.
   2. The coverslip will not pick up fungal elements if it is too dry or too wet. An additional mount should be made if the first attempt is unsuccessful.

B. Tease mount procedure
   1. Rigorous treatment of the hyphae does not preserve the original position of the structures of spores, conidiophores, etc.

C. It is important to remember that the edge of the colony represents the youngest portion and the center represents the oldest.
   1. If a mount is inadequate, a repeat mount can be done using material 8-10 mm from the colony periphery.

2. If only sterile hyphae are seen, this may be a result of the youth of the colony or that the fungus will not sporulate on the medium used to grow the mold.

X. References

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B. Koneman, EW, Roberts, GD, Wright SF. 1978. Practical Laboratory Mycology, 2nd ed. Williams and Wilkins Co., pp. 19-20.
C. Haley, LD, Carey, CS. 1978. Laboratory Methods in Medical Mycology. 4th ed. HEW publication no (CDC) 78-8361, pp 29-30.
D. Holmes, RL. 1992. Lactophenol cotton blue-PVA fungal touch preparation. Abstract C-139, 92nd Annual American Society for Microbiology meeting, New Orleans, LA.

XI. Document Control

- Microbiology Director Approval: Dr. Ann Robinson 5/19/2000
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