## LACTOPHENOL ANILINE BLUE (LPAB)

### Purpose

To determine the morphology of the conidiogenous cells and the conidia that they give rise to in order to identify a filamentous fungus.

### Principle

LPAB contains lactic acid as a clearing agent, phenol as a disinfectant, glycerol to prevent drying and Aniline Blue which is the dye that stains fungi. LPAB is a wet preparation.

### Procedure

**1) TEASE PREP**

The test must be performed in the Laminar Airflow Biosafety Cabinet. First, observe the gross morphology of the colony carefully to determine whether or not the culture is mouldy, granular or a mixture of both. It is important to prepare the LPAB preparation by "teasing" the fungus not "chopping" it.

Materials required for LPAB staining:

1. LPAB reagent
2. Probe to get the specimen
3. Teasing needles
4. Glass slides
5. Coverslips
6. Lead or wax pencils
7. Disinfectant bucket
8. Electric incinerator
9. Clear nail polish
10. Slide tray

**Tease Prep Procedure:**

1. Sterilize the loop and the needles in the incinerator and allow them to cool.

2. Label slide, place 2 drops of LPAB reagent on the slide.

3. Cut a small piece of the fungus from a granular or colored part of the colony, somewhere away from the central part towards the periphery and place the piece of the fungus in the LPAB in the upside down position.

4. Hold the thallus with the needle and gently tease the inverted side of the specimen into the staining (LPAB) fluid.

5. After enough teasing, remove all the solid particles and the agar from the mixture and discard in the disinfectant container.

6. Put a coverslip gently onto the LPAB preparation and hold the slide preparation briefly over the incinerator opening. Heating the slide will help to stain the cell wall of the fungi and kill the spores on the surface of the slide.

7. Seal the preparation with nail polish if necessary for a permanent mount.

8. Examine under the light microscope using the low power objective.

**2) SCOTCH TAPE PREP FROM PLATE CULTURE**
**(Not done on suspected dimorphic fungi)**

1. Place a drop of LPAB onto a clean glass slide.
2. Take a small piece of clear scotch tape and loop back on itself with sticky sides out.
3. Hold the tip of the loop securely with forceps.
4. Press the sticky side firmly to the surface of the fungal colony.
5. Pull the tape gently away from the colony.
6. Open up the tape strip and place it on the drop of LPAB on the glass slide, making sure that the entire sticky side adheres to the slide.
7. Examine under the light microscope.

## Reference


Davise H. Larone. Medically Important Fungi. A Guide to Identification. 3rd ed. 1995, ASM Press.

Martha E. Kern. Medical Mycology. 1985.