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LACTOPHENOL COTTON BLUE TECHNIQUE

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<http://www.youtube.com/watch?v=OMIF1Elr9i4&feature=plcp>

This document is a transcript of the above video.

The aim of the lactophenol cotton blue (LPCB) technique is to provide a quick and easy method of staining fungi for observation under the light microscope. It is a wet mount preparation – fungal samples are simply transferred onto a slide and viewed wet. The “cotton blue” is a histological stain which turns the fungal cell walls blue. Lactophenol helps preserve the fungal structures and kill any microorganisms. The LPCB also serves as a mounting medium for placing on a cover slip.

Steps:

1. A drop of Lactophenol solution is placed onto a clean glass microscope slide.
2. The needles for handling the fungus are sterilised using a Bunsen burner.
3. Hazard alert: safety precautions are necessary in the use of Bunsen burner while wearing plastic gloves.
4. Using one sterile needle a small amount of fungal culture is removed from the edge of the specimen – this will contain younger colonies.
5. The fungal culture is spread onto a slide by using second needle in order to tease out the fungal structures.
6. The needles are sterilised in a Bunsen flame after use.
7. The cover slip is gently placed on the slide by lowering it down and avoiding air bubbles.
8. The slide is now ready to be examined under the microscope.

9. Fungi may be detected by the presence of characteristic structures such as, micro/macroconidia, spores, and hyphae. These fungal elements differ depending on the fungal species.

Use the low power x10 objective to locate the object.

Use the high power x40 objective to confirm the presence of fungal structures.

Microscopic image of *Microsporium gypseum* at x40 power objective.



Reference illustrating use of LPCB in practice.

Leck A (1999). Preparation of lactophenol cotton blue slide mounts. Community Eye Health. 1999; 12(30): 24. Available 20th August 2012 at:

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1706009/>