

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MIMYC\10\06\v01	Page 1 of 3
Section: Mycology Bench Manual	Subject Title: Appendix VI - Slide Culture	
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APPENDIX VI

SLIDE CULTURE

I. Purpose

A slide culture is used to preserve and observe the natural state and the actual structure showing conidiogenous cells and conidia of the fungus.

Before setting up a slide culture, it is important to do an LPAB preparation and demonstrate that the organism is susceptible to Cycloheximide (Exception: Dermatophytes and *Sporothrix schenckii*).

Note: NEVER SET UP A SLIDE CULTURE ON A SUSPECTED *COCCIDIOIDES*, *BLASTOMYCES*, *PENICILLIUM marneffeii* AND *HISTOPLASMA* CULTURE OR ANY WHITE MOULD THAT GROWS WELL ON MYCOSEL.

II. Procedure

1. Place a sterile filter paper in a small petri dish.
2. Add some sterile distilled water.
3. Place 2 pieces of wooden sticks on the filter paper, a few centimetres apart.
4. Place a clean alcohol flamed heat sterilised glass slide on the sticks.
5. Using a sterile spatula, cut a small square (about 1 x 1 cm) of agar block from the Potato Dextrose Agar (PDA) (or 3% Salt Agar) and place it on the centre of the slide.
6. Using a needle or spatula, inoculate the agar with a small amount of fungus under test on each of the four sides of the block.
7. Place a heat-sterilized coverslip over the block and press down gently.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MIMYC\10\06\v01	Page 2 of 3
Mycology Bench Manual		

8. Incubate at 28°C or room temperature.
9. Examine **daily** for sufficient growth.
10. When good growth appears, place a drop of LPAB on a clean slide, remove the coverslip using forceps, pass the top side of the coverslip in front of the incinerator opening to fix and place it over the LPAB on the slide.
11. Examine under the light microscope. If the preparation shows sufficiently developed structures, prepare the second preparation from the slide by removing the agar block from the slide. Discard the agar in the sharps container. Place a drop of LPAB on the slide and a new coverslip on it. Both preparations can be preserved indefinitely by sealing the edges with nail polish.
12. If the fungus is still underdeveloped, add a fresh coverslip on the agar block, reseal the plate with parafilm and continue incubation.

III. Reference

1. Davise H. Larone. Medically Important Fungi. A Guide to Identification 1995, ASM Press.

Setting up Slide Culture

