# Slide Culture

## I. Principle

Slide cultures are used when the conidial configuration of a mold cannot be determined in a lactophenol cotton blue preparation. It is the best method to preserve and observe the reproductive structures of a fungus. It is not a rapid technique, but the fine points of the microscopic morphology of a mold can be studied. A slide culture should not be set up unless a LPCB prep has already been performed. This will avoid the set up of a slide culture on a dimorphic fungus.

## II. Specimen

A. Non- sporulating mold isolate

## III. Reagents and Material

A. Sterile water, store at room temperature until the stated expiration date.
B. Sterile (15 X 100 mm) petri dish with one piece of filter paper
C. Potato flake agar, store at 4°C.
D. Cornmeal agar, store at 4°C.
E. Glass slides
F. 22 X 22 mm and 24 X 50 mm coverslips
G. Lacto-phenol cotton blue, store at room temperature until the stated expiration date.
H. Sterile, disposable scapel
I. Fungal tape
J. Pencil
K. Marking pen
L. Forceps
M. Clear nail polish
N. Sterile 15 X 100 mm Petri plates
O. Microscope with 10X and 40X objectives

## IV. Safety

A. All cultures of filamentous fungi must be handled in the biological safety cabinet.
B. Slides cultures should **NOT** be set up on suspected dimorphic fungi (*C. immitis, B. dermatitidis, H. capsulatum*) to reduce potential exposure to these organisms.

## V. Procedure

A. Direct plate slide culture
    1. Obtain a potato flake agar plate.
    2. Streak the mold in three lines on the plate, creating a triangle pattern.
    3. Insert a flamed, sterile 22 X 22 mm coverslip into each streaked area at a 45 degree angle.
    4. Fungal tape and incubate the plate agar side down until growth occurs.

5. Remove one coverslip at a time. Place it on a glass slide with a drop of lactophenol cotton blue stain (LPCB). Place an additional drop of LPCB on top of the coverslip, and cover it with a 24 X 50 mm coverslip.
6. Examine the mount under 10X and 40X for characteristic microscopic morphology.
7. These slides may be sealed with clear nail polish and retained indefinitely.

B. Traditional slide culture
1. Obtain potato flake agar and cornmeal agar plates.
2. Moisten a piece of filter paper that is inside a Petri plate with no more than 1.5 mL of sterile water. The paper should be wet, but the plate should not contain excess water.
3. Label a glass slide with the patient's last name, date, and type of specimen, and place it in the bottom of the Petri plate.
4. Cut a small square of potato flake agar (about 2 cm X 2 cm) with a scalpel, and place it on the slide. Repeat this procedure with the cornmeal agar.
5. Cut 8 small fragments of the fungus colony approximately 4-5 mm in from the colony periphery, and inoculate the edges of the agar squares.
6. Place flamed 22 x 22 mm coverslips over the agar squares, and apply slight pressure to insure adherence.
7. Replace the Petri plate cover, and fungal tape the plate.
8. At the same time, inoculate additional potato flake agar and cornmeal agar plates with the colony. If adequate sporulation has not occurred on the slide cultures in 14 days, reset new slide cultures using the growth from the potato flake agar and cornmeal agar plates. The plates will also demonstrate any pigment production.
9. Examine the slide cultures periodically for growth under low power (10X) to determine when characteristic sporulation occurs. Do this by removing the preparation from the moist chamber, drying the bottom of the slide with a tissue, and placing it on the stage of the microscope. The fungus will grow on the surface of the slide and on the undersurface of the coverslip.
10. When sporulation is well developed, under the biosafety hood, carefully remove the coverslip with forceps, and place it on a drop of LPCB on a second slide. Add another drop of LPCB, and place a 24 X 50 mm coverslip on top.
    a. A new cover slip may be put on the agar block for additional sporulation, or the agar block may be discarded into an autoclave bag, and place a drop of LPCB on the original slide and coverslip it.
11. These slides can be sealed with clear nail polish and retained indefinitely.
12. Examine the preparations under 10X and 40X for characteristic morphology.

## VI. Interpretation

A. Observe the mount for intact characteristic microscopic fungal reproductive structures and a blue staining appearance
B. Identifications are based on macroscopic colony morphology and microscopic characteristics (refer to **"Culture Evaluation"** procedure).

## VII. Result Reporting

A. All identifications resulting from the slide cultures should be brought to the attention of the supervisor and the Microbiology doctor.
B. Rounds will determine how isolates should be reported.

## IX. References

A. Larone, DH. 1995. Medically Important Fungi: A Guide to Identification. American Society for Microbiology, Washington DC, pp 215-217.
B. Koneman, EW and Roberts GD. 1985. Practical Laboratory Mycology, 3rd edition. Waverly Press, Inc., p 51-52.

## X. Document Control

Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010.
Microbiology Director Approval: Dr. Ann Robinson 05/19/2000
Microbiology Supervisor Reviews: Jerry Claridge 06/2001, 04/2002, 03/2003, 04/2004, 07/2005, 06/2006, 06/2007, 05/2008, 07/2009, 04/01/12, 03/2013, Jason Ammons 07/2015
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