MMTN
MEDICAL MYCOLOGY
TRAINING NETWORK

Techniques of setting up slide culture and preparation of slides for examination

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ASIA FUNGAL WORKING GROUP
an ISHAM working group

Slide culture for mold identification

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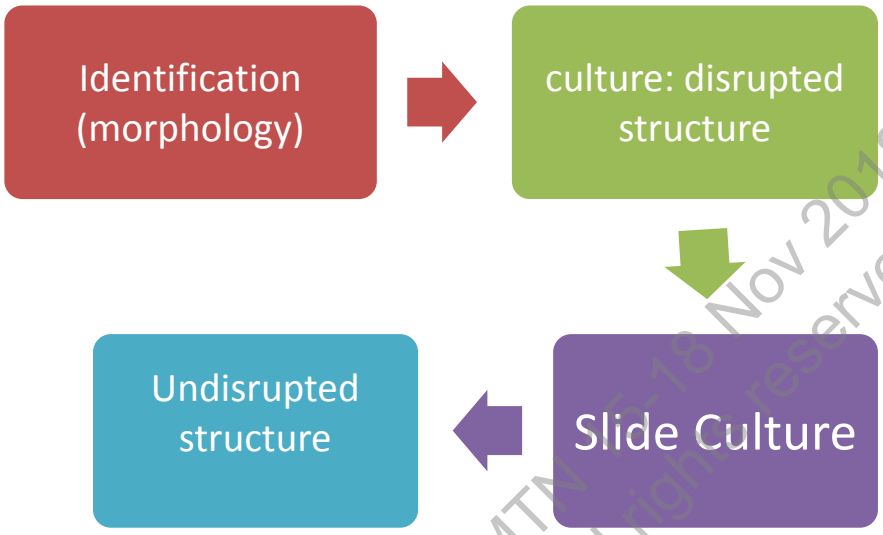
Mould identification

- identification of moulds is mostly based on their morphology
- based on hyphae formation and sporulation, but
- materials taken directly from the primary culture often cause structural damage & morphological observation becomes inaccurate

Slide culture

1. A thin, one layer culture, enable us to learn on morphology
2. Precise arrangement of conidiophores & sporulation
3. (2) are important in the morphology based species identification

Problem in fungal identification



Modified from:
Riddell RW. Mycologia 1950;42(2):265-70
http://vlab.amrita.edu/?sub=3&brch=76&sim=693&cnt=2

SLIDE CULTURE USING NORMAL GLASS

Materials needed

- Primary culture, 7-10 days old
- Sterile Sabouraud dextrose agar in Petri dish
- Sterile Petri dish & filter paper
- Sterilized U-shaped glass rod or glass slide
- Sterilized Glass slides & coverslips
- Lactophenol cotton blue (LPCB) stain or lactophenol stain (LP)
- Sterile scalpel & inoculating needle/loop wire
- Sterile distilled water
- 95% ethanol
- Sterile forceps
- flames

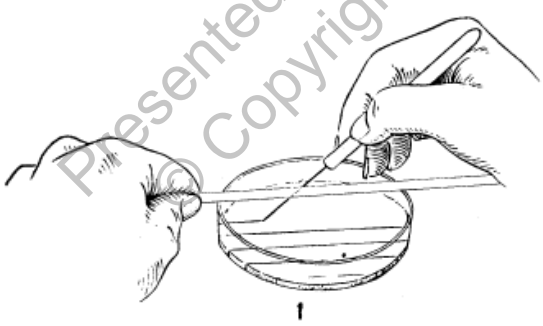
Slide Culture Preparation

- Aseptically, with a forceps, put sterile filter paper in a sterile Petri dish.
- Place a sterile (U-shaped) glass rod on the filter paper (rod can be sterilized by flaming & held by forceps) or a pile of sterile glass slide (3-4 glass slide) instead of glass rod
- Pour sterile dist. H_2O on filter paper, just enough to completely moisten it.
- With flamed sterile forceps, place a sterile slide on the U-shaped rod/ pile of sterile glass slide
- Gently flame a scalpel to sterilize, and cut a 0,5-1 cm square block of the medium from the plate of Sabouraud's agar.
- Pick up the agar block by a scalpel & place onto the center of the slide inside the petri dish

Slide Culture Preparation

- Using flamed sterile loop wire, inoculate four sides of the agar square with spores or mycelial fragments of the fungus. Cool the loop prior to picking up spores/hyphae.
- Aseptically, place a sterile cover slip on the upper surface of the agar cube.
- Place the cover on the Petri dish & incubate at RTfor 48 hours or >.
- After 48 hours, examine the slide under low power magnifications.
- If growth has occurred there will be growth of hyphae & production of spores. If growth is inadequate & spores are not evident, allow to grow for another 24–48 hours before making the stained slides.

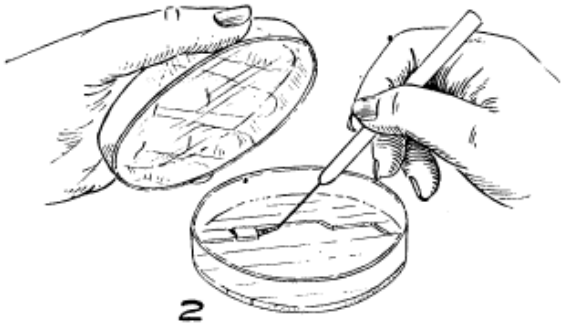
Slide Culture



About 10 ml of mostly SDA is melted and poured into a sterile 9 cm Petri dish to form a layer 2 mm deep.

Solidifies the medium at room temperature or cooling by refrigerator, 0.5 -1 **cm squares are ruled out** over the whole plate using a (flamed) sterile dissecting needle or (flamed) sterile scalpel

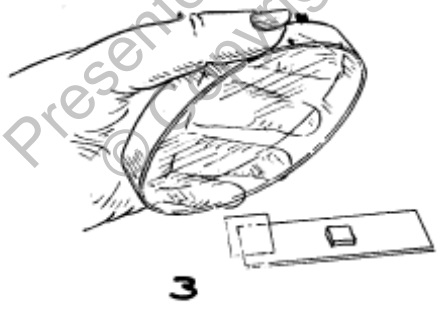
Slide Culture



An **agar square is now lifted out** of the Petri dish with precautions to prevent contaminations

Use sterile scalpel/loop wire

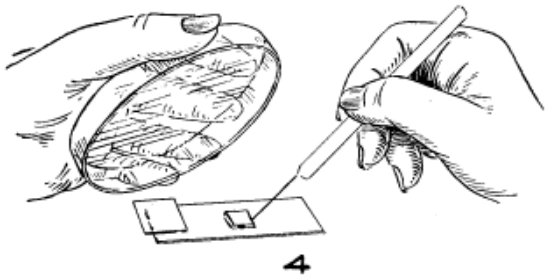
Slide Culture



transferred rapidly to the **center of the cooled slide**

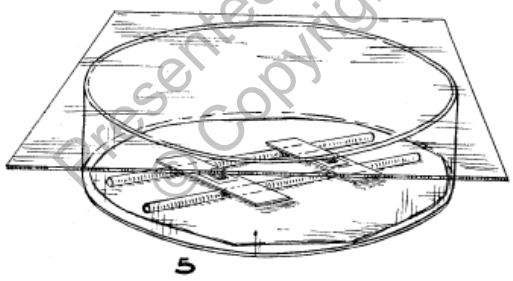
Better: if the glass slide has been placed in a sterile petri dish before placing agar square. It will reduces contamination

Slide Culture



Using sterile **loop/needle wire small inoculum** of spores, or a small bit of mycelium, **is placed at the center of each edge** of the agar block over the whole 2 mm depth at this point

Slide Culture

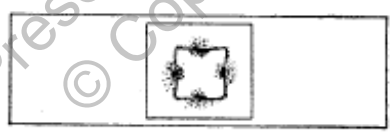


- With sterile forceps the sterile **cover slip** is placed centrally upon the **upper surface of the agar square**,
- the slide is then **transferred to a moist chamber** i.e. sterile petri dish with glass rod or a pile of glass slide (place the slide perpendicular to the length of the pile of glass slide)

Slide Culture

- Add some drops of sterile distilled water to the petri dish to keep the culture moist or you can do it at the beginning of the process
- Put the cover of petri dish & incubate at room temperature for a view days
- Check under the microscope to observe the grow of the fungus.
- Additional incubation time if necessary

Slide Culture



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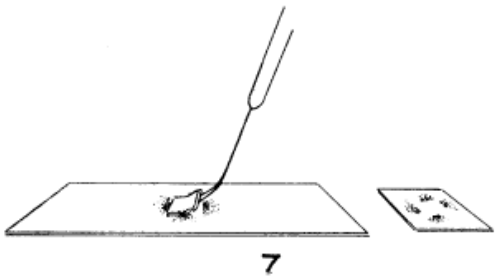
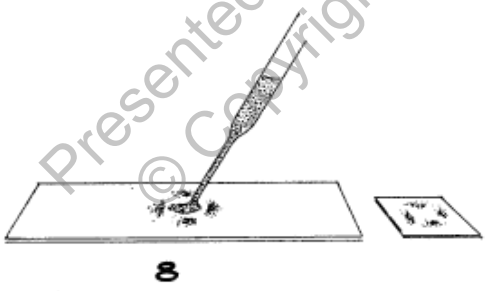
- Growth termination/making the slide: do it before excessive sporulation
- **growths of about 1 mm wide give the best results**

Slide Culture



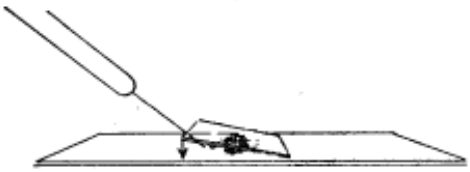
- The **cover slip is lifted** vertically from the agar block, placed it uppermost
- For the agar which remain in the glass slide a sterile dissecting needle/loop wire is inserted under the agar to lift. Then the **agar block is discarded leaving** a thin layer culture in the glass slide
- Now we have two slides culture, i.e. from the cover slip & the glass slide

Slide Culture



A drop of 70-95 % ethyl alcohol is applied to the center of each of the rings of growth in cover slip & glass slide to eliminate air trapped.

Slide Culture



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- Just before the alcohol has completely evaporated a drop of **lactophenol cotton blue** (LPCB) was applied to clean glass slide. Then a **slowly the cover slip with colony is put on the** glass slide **with LPCB.**
- Similar method was done on colonies in glass slide, then a clean cover slip is put slowly (to avoid air trapped) on the surface of the colony which had been given alcohol & LPCB.
- The slides now is ready for investigation under the microscope. Start with 100× magnification continued by 400 × magnification

Modified from: Cappucino. Microbiology: A Laboratory Manual 11th ed. pp.251-3

SLIDE CULTURE USING CONCAVE GLASS

Slide Culture using concave glass slide

1. Take sterile (empty) petri dish
2. Put sterile filter paper
3. Put a sterile bent glass rod or two glass rod/pile of glass slide on the filter paper.
4. Add drops of sterile dist. water to dampen the filter paper, just enough to make it completely damp.
5. Using forceps dip the concave glass slide & cover slip to 70-95% ethyl alcohol, pass through Bunsen burner flame, remove from flame, and hold until all the alcohol has burned off the slides and coverslips.

Slide Culture using concave glass slide

6. Cool slides and cover slips. Place a slide, concave side up, with a coverslip to one side of the concavity, on the glass rod in the Petri dish.

7. With a toothpick, add petroleum jelly to three sides surrounding the concavity of each slide. The fourth side will serve as a vent for air.

Slide Culture

8. With a sterile Pasteur pipette, add one or two drops of cooled Sabouraud agar (ca,. 45˚C) to the concavity of the slide.

9. Place a coverslip over the concave portion of each slide so that it is completely sealed.

10. With forceps, stand the slide upright inside Petri dish until the agar solidifies, as illustrated below:

Slide Culture

11. When agar is fully hardened, slide coverslips downward with forceps, and with a sterile needle inoculate the slide with the spores/mycelium from the test cultures.

12. Push the coverslips to their original positions, thereby sealing off the slide.

13. Remoisten filter paper when necessary during the incubation period.

Slide Culture

14. Place the slide on the U-shaped bent rod/rods, put Petri dish cover,

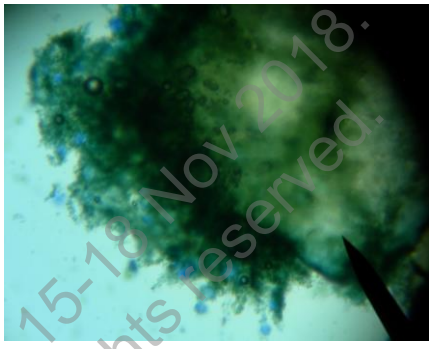
15. Incubate the preparations for 2-7 or more days at 25°C.

RESULTS OF SLIDE CULTURE

Slide culture Aspergillus

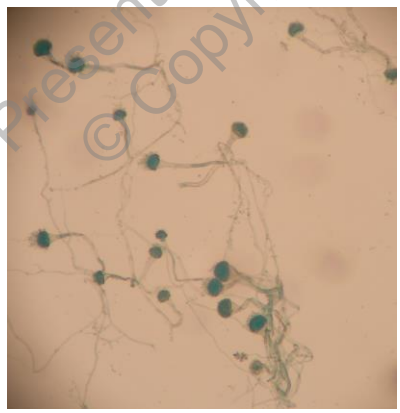
Culture

Microscopy - LPCB



Results- LPCB

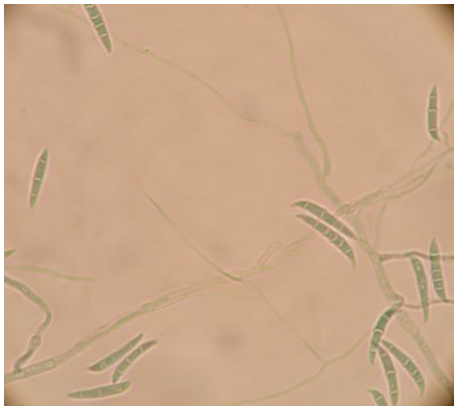
Slide culture, 100× magnification



Slide culture, 400× magnification

Slide culture Fusariumsp

Fusarium sp. with Lacto phenol



notes

- Without LPCB we can see the original colour of the fungus.
- See the hyphae & the specific conidia

A. fumigatus

Thank you very much

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