LESSON ASSIGNMENT

LESSON 10
Maintaining Stock Cultures.

TEXT ASSIGNMENT
Paragraphs 10-1 through 10-6.

TASK OBJECTIVES
After completing this lesson, you should be able to:

10-1. Identify principles for maintaining a "working" stock culture.

10-2. Identify procedures used to recover organisms from a stock culture.

SUGGESTION
After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.
LESSON 10

MAINTAINING STOCK CULTURES

10-1. INTRODUCTION

a. Several reasons for maintaining a fungal stock culture collection exist, the most important of which is the need to maintain quality control of media. A stock culture collection is also a valuable source of reference cultures for a medical technologist who must identify atypical or unfamiliar fungal isolates on a daily basis. The ability to refer to known cultures, and to compare their properties with those of unknown isolates, is often of great advantage. Finally, laboratories in which new technicians are trained in diagnostic medical mycology must have a stock culture collection that represents, at the least, those fungi most frequently isolated from clinical specimens.

b. However, keeping a set of stock cultures on the shelf is insufficient. Keeping those cultures sporulating, and demonstrating characteristic colonial morphologies, is required to maintain a collection. Showing a new technician a white, woolly, non-sporulating, culture, the back of which is light brown, and expecting him to recognize it as an isolate of *Trichophyton rubrum* is unsatisfactory. Two major procedures are required to maintain a stock fungal culture collection properly. The first is to keep the organism viable until it is needed, and second is to retain typical colonial and microscopic morphologies of each fungus.

10-2. MAINTENANCE OF "WORKING" STOCK CULTURES

a. Some mycology laboratories that have continuous training programs must maintain stock cultures ready for use on short notice. These cultures are called "working" stocks.

b. "Working" stock cultures must be subcultured at regular intervals (four to six weeks) and checked for presence of spores and for development of sterile, pleomorphic mycelium. When pleomorphism develops, great effort must be made to avoid overgrowth of the typical mycelium by pleomorphic portions or the entire culture will be lost.

c. Several media may be required to maintain a culture that is typical and sporulating. Some fungi require alternating between two or three media to maintain their characteristic gross and microscopic morphologies.

d. The following table summarizes media used to maintain "working" stocks in the Mycology Training Branch at Center Disease Control. It should be noted that each stock culture is checked frequently for sporulation (Table 10-1).
### Table 10-1. Maintenance of "working" stock fungus cultures (continued)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsporum audouini</td>
<td>Sab. (1)</td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>d.s. (2); every third subculture should include Sab.</td>
</tr>
<tr>
<td>Microsporum gypseum</td>
<td>Rotate between Pd (3) and d.s.</td>
</tr>
<tr>
<td>Microsporum cookei</td>
<td>Sab.; if becoming pleomorphic, rotate between Sab. and d.s.</td>
</tr>
<tr>
<td>Microsporum persicolor</td>
<td>Sab.; may occasionally rotate with G-7 (6) or BL-S (4)</td>
</tr>
<tr>
<td>Microsporum nanum</td>
<td>Sab.; may occasionally rotate with d.s.</td>
</tr>
<tr>
<td>Microsporum distortum</td>
<td>Rotate between Sab. and BL-S, and medium # 1 (5)</td>
</tr>
<tr>
<td>Microsporum ferrugineum</td>
<td>Sab.</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>Rotate between Sab., BL-S, and medium # 1</td>
</tr>
<tr>
<td>var. interdigitale</td>
<td></td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>Keep on d.s. for spores; rotate between d.s., Pd or Sab. or G-5.5 (7)</td>
</tr>
<tr>
<td>var. mentagrophytes</td>
<td></td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>Rotate between Pd and medium # 1; enhancement of pigment on G-7</td>
</tr>
</tbody>
</table>

1. Sab. — Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
2. d.s. — Dilute Sabouraud dextrose agar
3. Pd — Henrici's potato dextrose agar
4. BL-S — Sabouraud dextrose agar, polypeptone, pH 7.0, Bioquest
5. Medium #1 — Trichophyton medium #1, Difco
6. G-7 — Mycobiologic agar, pH 7.0, Gibco
7. G 5.5 — Sabouraud agar, pH 5.5, Gibco
8. S+T — Sabouraud agar, pH 5.5, Difco + Thiamine
9. BHI — Brain Heart Infusion agar
<table>
<thead>
<tr>
<th>Fungus</th>
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</tr>
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<tbody>
<tr>
<td>Trichophyton tonsurans</td>
<td>Sab., rotate occasionally with S+T (8)</td>
</tr>
<tr>
<td>Trichophyton terrestre</td>
<td>Sab. or Pd; check sporulation</td>
</tr>
<tr>
<td>Trichophyton ajelloi</td>
<td>BL-S and Sab.; d.s. to maintain sporulation</td>
</tr>
<tr>
<td>Trichophyton verrucosum</td>
<td>S+T</td>
</tr>
<tr>
<td>Trichophyton schoenleinii</td>
<td>Sab.</td>
</tr>
<tr>
<td>Trichophyton concentricum</td>
<td>Sab.</td>
</tr>
<tr>
<td>Trichophyton violaceum</td>
<td>Rotate between Sab. and S+T (Sab --&gt; S+T; S+T --&gt; Sab.); both media used at all times</td>
</tr>
<tr>
<td>Trichophyton megninii</td>
<td>Sab.; BL-S occ. for better pigment</td>
</tr>
<tr>
<td>Trichophyton soudanense</td>
<td>Sab.</td>
</tr>
<tr>
<td>Trichophyton gallinae</td>
<td>Rotate between Sab. and S+T (see Trichophyton violaceum)</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>Sab.; occasionally rotating with BL-S may enhance sporulation but causes gross morphology changes; culture may die if kept on BL-S</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>Pd; occasionally rotate with 18% V-8 agar to enhance sporulation</td>
</tr>
<tr>
<td>Fonsecaea pedrosoi</td>
<td>Same as Cladosporium</td>
</tr>
<tr>
<td>Phialophora verrucosa</td>
<td>Same as Cladosporium</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>Pd</td>
</tr>
</tbody>
</table>

1. Sab. - Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
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5. Medium #1 - Trichophyton medium #1, Difco
6. G-7 - Mycobio logic agar, pH 7.0, Gibco
7. G 5.5 - Sabouraud agar, pH 5.5, Gibco
8. S+T - Sabouraud agar, pH 5.5, Difco + Thiamine
9. BHI - Brain Heart Infusion agar

Table 10-1. Maintenance of "working" stock fungus cultures (continued).
<table>
<thead>
<tr>
<th>Fungus</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histoplasma capsulatum</td>
<td>Pine's mycelium medium and yeast-form medium</td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
<td>Sab. for mycelium; BHI (9) for yeast</td>
</tr>
<tr>
<td>Paracoccidioides brasiliensis</td>
<td>Same as for B. dermatitidis</td>
</tr>
<tr>
<td>Coccioides immitis</td>
<td>Sab.</td>
</tr>
<tr>
<td>Candida, Cryptococcus, Torulopsis and Trichosporon spp.</td>
<td>Yeast malt extract agar; Sab. is satisfactory</td>
</tr>
<tr>
<td>Geotrichum spp.</td>
<td>Sab. or Pd</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>Pd</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>Pd</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>Sab. for color; Pd for sporulation</td>
</tr>
<tr>
<td>Chrysosporium spp.</td>
<td>Pd; rotate occasionally with Sab.</td>
</tr>
<tr>
<td>Sepedonium spp.</td>
<td>Rotate between Sab. and Pd</td>
</tr>
<tr>
<td>Scopulariopsis spp.</td>
<td>Pd</td>
</tr>
<tr>
<td>Nocardia spp.</td>
<td>7H10 or 7H11</td>
</tr>
<tr>
<td>Actinomadura spp.</td>
<td>7H10 or 7H11</td>
</tr>
<tr>
<td>Streptomyces spp.</td>
<td>Rotate between 7H10, Sab. and BHI; isolates vary with preferences for these media</td>
</tr>
</tbody>
</table>

1. Sab. - Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
2. d.s. - Dilute Sabouraud dextrose agar
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Table 10-1. Maintenance of "working" stock fungus cultures (concluded).
10-3. PLACING STOCK CULTURES IN STERILE DISTILLED WATER

An excellent method of maintaining stock cultures is to place fungal cultures in sterile, distilled water. Two groups of organisms that can easily be entered into water stocks and recovered when needed are yeasts and aerobic actinomycetes. Entering mold cultures into water requires dexterity on the part of the technologist.

a. Placing Yeast Cultures into Water.

(1) Inoculate yeast to a Sabouraud or yeast malt extract agar slant. Incubate at room temperature for 48 to 96 hours.

(2) Add approximately 4 mL of sterile, distilled water to the slant.

(3) Gently agitate the slant on a mechanical mixer. When most of the growth on the slant has gone into suspension, draw off aqueous suspension and place in a small, sterile, screw-capped vial or a small, screw-capped test tube.

(4) Ring cap with parafilm and store container in a dust-free area at room temperature, or at 20ºC. Most stocks remain viable for four or five years.

b. Placing Actinomycetes and Mold Cultures in Water.

(1) Inoculate one or two tubes of 7H10, potato dextrose or Sabouraud agar, depending upon the organism. For heavily sporulating cultures, such as Aspergillus and Penicillium species, or for an actinomycete, only one tube of medium is necessary; molds that rarely sporulate or do not sporulate heavily usually require two tubes. If the aqueous suspension is less than a MacFarland #4, the culture may not survive for long periods.

(2) Incubate slants at room temperature. Usually five to ten days is adequate for most mold cultures; actinomycetes may require 14 to 20 days (H. capsulatum, B. dermatitidis, and C. immitis are cultured at room temperature and stored on slants in a sealed container at 4ºC to 10ºC).

(3) Add approximately 5 mL of sterile distilled water to each slant.

(4) Sterilize one end of an approximately 8-inch-long, rounded-end, stainless steel, micro spatula in a Bunsen burner flame. Cool by inserting into a tube of sterile agar. Gently scrape all surface growth accumulated on the slant into the water, taking care not to remove any agar.

(5) Flame mouth of the culture tube and the sterile vial. aqueous suspension into the vial.

(6) Seal and store, as outlined in para a (4) above.
10-4. PLACING STOCK CULTURES UNDER OIL

If handled properly, stock cultures placed under oil will remain viable for at least ten years.

a. Sterilize approximately 100 mL of heavy mineral oil in a 250 mL flask by autoclaving at 15 psi for 45 minutes to one hour. (Oil that appears cloudy after sterilization has absorbed water during autoclaving. Do not use.)

b. When mold cultures have begun to sporulate (usually 5 to 10 days), or yeast and actinomycete cultures are well developed, on the agar slants, flame the mouths of the flask and the culture tube.

c. Gently pour sterile oil over the entire agar slant. (Agar not covered by oil will act as a wick, enhancing water evaporation rate from the agar slant.)

d. Screw down the cap of the tube down and store in a dust-free area, at room temperature.

10-5. RECOVERING ORGANISMS FROM WATER STOCK

a. Place approximately 0.1 mL of sediment on the surface of a Sabouraud agar plate.

b. Streak inoculum across the agar as is done for a bacterial culture.

c. Incubate culture at room temperature, agar surface up.

d. When growth becomes obvious, examine all areas of plate for colonies characteristic of the fungus. Some pleomorphism will develop in the water stock culture and usually will appear as rapidly growing white colonies. To isolate typical colonies from the pleomorphic ones requires adequate surface area; hence, plates are used rather than agar slants.

e. Pick two or three typical colonies, inoculating each to an agar Incubate at room temperature slant.

10-6. RECOVERING ORGANISMS FROM OIL SHOCK

Many molds and yeasts develop sterile aerial hyphae when placed under oil. It is essential to avoid these hyphae when recovering a culture from oil stock.

a. Flame the mouth of the stock culture tube and pour oil into a discard container. As oil is flowing into the container, take a sterile probe and dislodge any aerial mycelium present in the culture. Allow it to flow out with the oil. The portion of the fungal colony to be subcultured will adhere to the surface of the agar slant.

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b. When oil has been decanted, continue to hold the tube at an angle and, with a probe, remove some of the fungal colony and transfer to a Sabouraud agar plate.

c. If possible, break the inoculum into small portions with a probe and distribute over the agar surface.

d. Incubate cultures at room temperature, agar surface up.

e. Subculture several typical colonies to agar slants.

Continue with Exercises
EXERCISES, LESSON 10

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Name three reasons for maintaining stock fungus cultures.

________________________________________
________________________________________
________________________________________

2. The purpose of growing "working" stock cultures is to:
   a. Maintain readily available stock cultures.
   b. Monitor stock cultures for pleomorphism.
   c. Alternate types of media for the culture.
   d. Combine more than one organism in a stock culture.

3. What two media are used to maintain "working" stock culture of *Microsporum gypseum*?

________________________________________
________________________________________

4. Two methods for long-term maintenance of stock cultures are:

________________________________________ and ____________________________________
5. How long can stock cultures placed in sterile distilled water be expected to remain viable?
   a. 1 month.
   b. 1 year.
   c. 5 years.
   d. 10 years.

6. Organisms easily stored in sterile, distilled water include:
   __________________________ and _________________________.

7. To maintain stock cultures under oil, sterile heavy mineral oil is poured over the agar slant:
   a. Immediately after it has been inoculated.
   b. So that the entire agar slant is covered.
   c. To a depth of 1/2 inch.
   d. After inoculation, and periodically thereafter.

8. To recover organisms from water stock:
   a. Streak 0.1 mL sediment on Sabouraud agar.
   b. Pour 0.1 mL supernatant water on Sabouraud agar.
   c. Inoculate typical colonies on a second Sabouraud plate.
   d. Incubate Sabouraud plate, reverse side facing up.
9. To recover organisms from oil stock, it is essential to:
   a. Inoculate a Sabouraud plate with an aliquot of overlaying oil.
   b. Avoid hyphae that have developed under oil.
   c. Inoculate portions of colony and oil on separate plates.
   d. Suspend portions of culture in sterile water for inoculation.

Check Your Answers on Next Page
SOLUTIONS TO EXERCISES, LESSON 10

1. Quality control of media; reference for identification of unknown isolates; training new personnel (para -la).

2. a. (para 10-2a)

3. Potato dextrose agar and dilute Sabouraud dextrose agar. (Table 10-1).

4. Placing in sterile, distilled water and placing in sterile, mineral oil. (paras 10-3 and 10-4)

5. c (para 10-3)

6. Yeast, aerobic actinomycetes. (para 10-3)

7. b (para 10-4)

8. a (para la-5a)

9. b (para 10-6)

End of Lesson 10