

General Microbiology Lab

Lab Session 2 - Culture Techniques

Introduction

Microbiologists need techniques to cultivate or grow microorganisms in the lab. First, they need specific culture media to grow the microorganisms they are interested in. They also need techniques to isolate or separate the diversity of microorganisms that often occur in a natural sample. The purpose of this lab is to become familiar with culture media and develop the proficiency necessary to isolate microorganisms from their natural environment, grow them in the lab, and then transfer them between various types of growth or culture media.

A. Review the following for reference:

1. Lab manual:

Exercise 10: Pgs 63-66. Note especially Figs 10.2, 10.3, 10.4 and 10.5

Exercise 11: Pgs 71-74. Note especially Figs 11.1 and 11.2.

Exercise 12: Pg 77.

2. Text: Chapter 5: Pgs 110-117.

3. Pay particular attention to the following: Kinds of cultures (pure vs. mixed); Kinds of culture media (agar vs. broth, defined vs. complex, selective vs. differential); and, Manipulation techniques: (streak plates, transfer techniques, aseptic techniques).

B. Do the following during lab:

-Work in groups of 3-4. Groups of 3 will have to organize to get all 4 mo's completed for 1a, 2a, 2b, 2c, and 3.

- Microorganisms are provided on slants. Choose microorganisms so that each group has all of the following:

Organism	Media to Inoculate*	Incubation temp.	Inc. time
<i>Bacillus subtilis</i>	TSB/TSA	30 C	24 hr
<i>Staphylococcus epidermidis</i>	TSB/TSA	37 C	24-48 hr
<i>Escherichia coli</i>	TSB/TSA	37 C	24-48 hr
<i>Neisseria sicca</i>	BHIA/BHIB	37 C	48-72 hr

*TSA/B = Trypticase Soy Agar/Broth. BHIA/B = Brain Heart Infusion Agar/Broth.

1. Streak plates: Each individual will need 3 plates and perform:

a. One streak plate with one of the supplied microorganisms.

Use the culture media, incubation temperature and time required.

b.

c. Two streak plates with your throat culture.

Inoculate Trypticase Soy Agar. Incubate at 37 C for 48 hours.

2. Transfers: Each individual will need one uninoculated broth tube and one uninoculated slant:

a. Transfer your 'chosen' mo from the inoculated slant provided to an uninoculated broth tube.

b. Transfer from the broth tube (in '2a' above) to an uninoculated slant.

c. Transfer from the inoculated slant (in '2a' above) to the newly inoculated slant in '2b' above.

3. Transfers: Work as a group. Each group will need one uninoculated slant for each mo.

Transfer each of the following mo's to a slant of culture media and incubate as directed:

Organism	Media to Inoculate*	Incubation temp.	Inc. time
<i>Saccharomyces cerevisiae</i>	TSA	30 C	48 hr
<i>Corynebacterium xerosis</i>	BHIA	37 C	48 hr
<i>Streptococcus salivarius</i>	BHIA	37 C	48 hr
<i>Pseudomonas fluorescens</i>	TSA	30 C	48 hr

4. Observe and describe the mo's on the demonstration microscope slides.

C. Techniques:

1. The basic method for doing a streak plate is as follows:

D. Results:

Demonstration Slides

1. *Neisseria gonorrhoea* (pus smear)

2. *Mycobacterium tuberculosis* (sputum sample)

3. *Bacillus anthracis* (tissue [liver?] sample)

4. *Streptococcus pneumoniae* (pure culture)

5. *Treponema pallidum* (chancre smear)

6. Oral or Rectal smear

E. Discussion and Conclusions