

# Microbiology for the Health Sciences Laboratory

## Lab Session 3 - 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 particular sample or specimen. 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. Introduction: Work carefully and try to develop good techniques as you culture microorganisms.
2. Text: Chapter 4: Especially pages 85-87 and 94-97.
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:

##### Part A: Isolation and Transfers

Work in groups of 3. Microorganisms are provided on slants.

Supplied Organisms: Group A	Media to Inoculate*	Incubation Temperature	Incubation Time
<i>Bacillus subtilis</i>	TSB & TSA	30 C	24 hr
<i>Staphylococcus epidermidis</i>	TSB & TSA	37 C	24 hr
<i>Escherichia coli</i>	TSB & TSA	37 C	24 hr

\*TSA = Trypticase Soy Agar. TSB = Trypticase Soy Broth.

1. Streak plates: Work alone.  
Choose microorganisms so that your group has one set of the mo's from Group A.  
Each individual will need:  
Choose 1 of the supplied Group A mo's. You will need 3 culture plates of TSA.
  - a. Do one streak plate with one of the supplied microorganisms listed above.  
Use the culture media, incubation temperature and time required.
  - b. Do two streak plates with your throat culture.  
Inoculate Trypticase Soy Agar plates. Incubate at 37 C for 24 hours.
  - c. Optional: Do a streak plate with your environmental sample if you did this last week.
2. Transfers: Work alone.  
Choose microorganisms so that your group has one set of the mo's from Group A.  
Each individual will need the following:  
1 supplied organism on a slant (x), 1 uninoculated broth tube (y) and 1 uninoculated slant (z)  
Each individual will do the following in order:
  - a. Transfer your 'chosen' microorganism from the inoculated slant (x) to the uninoculated broth tube (y).
  - b. Transfer an inoculum from the broth tube (y) to the uninoculated slant (z).
  - c. Transfer an inoculum from the inoculated slant (x) to the newly inoculated slant (z).
3. Transfers: Work as a group.  
Each group will need one inoculated BHIA slant for each microorganism in Group B below.  
Transfer each of the following mo's to a slant of culture media and incubate as directed:

Supplied Organisms: Group B	Media to Inoculate*	Incubation temp.	Inc. time
<i>Neisseria sicca</i>	BHIA	37 C	48 hr
<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

##### Part B: Demonstration Slides

1. Work on your own. 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:

2. The basic technique for transferring mo's is as follows:

**D. Results:**

Demonstration Slides

1. *Neisseria gonorrhoea* (pus smear)

---

2. *Mycobacterium tuberculosis* (sputum sample)

---

3. *Bacillus anthracis* (tissue sample)

---

4. Rectal smear

---

5. Oral smear

---

6.

---

**E. Discussion and Conclusions**

