

# BIO 201 Lab 4 Experiment 5 & 6

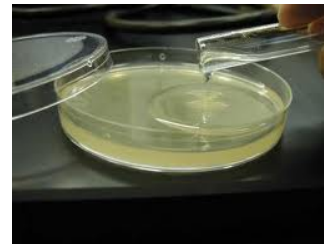
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# Overview

- I. **Exp. 5: Preparation of Culture Media**
- II. **Exp. 6: Standard Plate Counts**

# I. Exp. 5: Preparation of Culture Media

- ▶ **Purpose:** To learn about different types of culture media and how it is sterilized
- ▶ **Culture Media:** food source for bacteria, molds & yeasts
- ▶ **Composition:** varies
- ▶ **2 Physical Forms:**
  - Solid—contains agar
  - Liquid or broth—no agar



# I. Exp. 5: Preparation of Culture Media

## ▶ Agar

- Polysaccharide (galactose)
- Derived from marine algae or seaweed
- Solidifying agent—provides no nutrients
- Dissolves at 100°C/Hardens at 42°C
- Can be used in the food industry (carrageenan)
  - Thickener or emulsifier



# I. Exp. 5: Preparation of Culture Media

## ▶ Autoclave: sterilizer

- “Steam under pressure”
- Standard temperature–121°C,  
pressure–15 psi  
and time–15–20 minutes



- Do you have a form of an autoclave at home?
  - Home pressure cooker



# Overview

- I. **Exp. 5: Preparation of Culture Media**
- II. **Exp. 6: Standard Plate Counts**

## II. Exp. 6: Standard Plate Count

- ▶ **Purpose:** To determine the number of bacteria in a sample.
- ▶ **Quantitative procedure:** number of bacteria in a sample (solid or liquid)
- ▶ **Not applicable for molds.** Why? Multicellular.
- ▶ Sample needs to be **diluted** in sterile water in order to get a countable plate.
- ▶ **Countable Plate:** 30–300 bacterial colonies



## II. Exp. 6: Standard Plate Count

- ▶ Each colony is assumed to have arisen from one cell
- ▶ Procedure **not useful** in clinical microbiology
- ▶ Useful when testing consumer products to verify that they meet their claims





## **II. Exp. 6: Standard Plate Count**

- ▶ **General Steps: Standard Plate Count (Pour Plate Method)**
  1. Dilute specimen to get a countable plate
  2. Add diluted specimen to an empty plate
  3. Pour culture media; mix gently; let dry
  4. Incubate
  5. Count plates: determine the number of bacteria in the original specimen.

## II. Exp. 6: Standard Plate Count

### ► Pour Plate Method

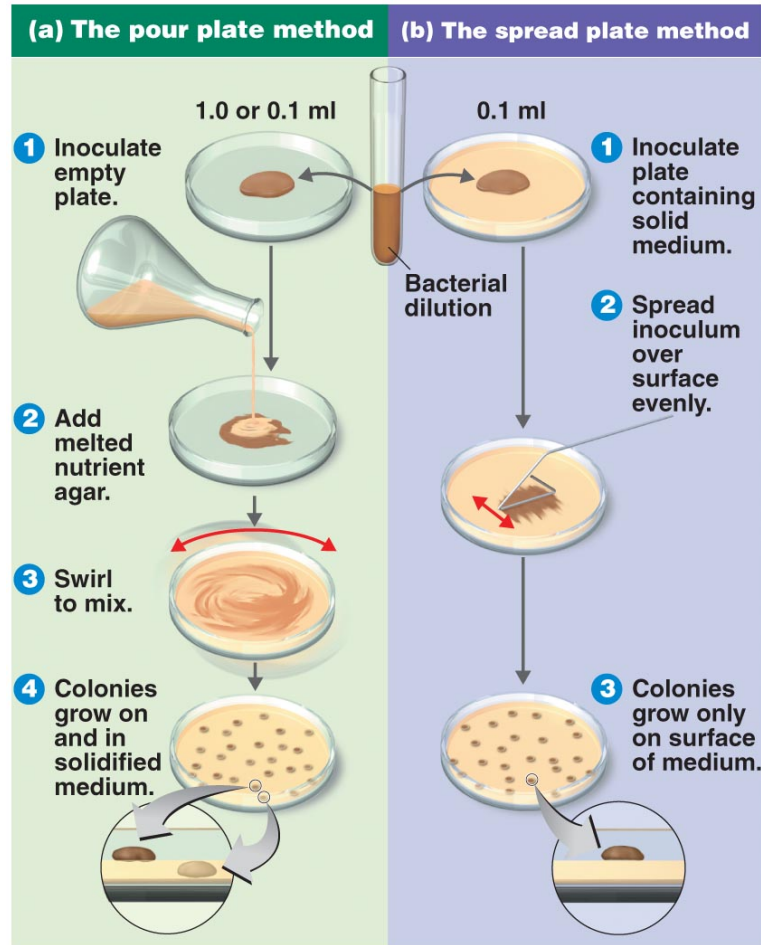
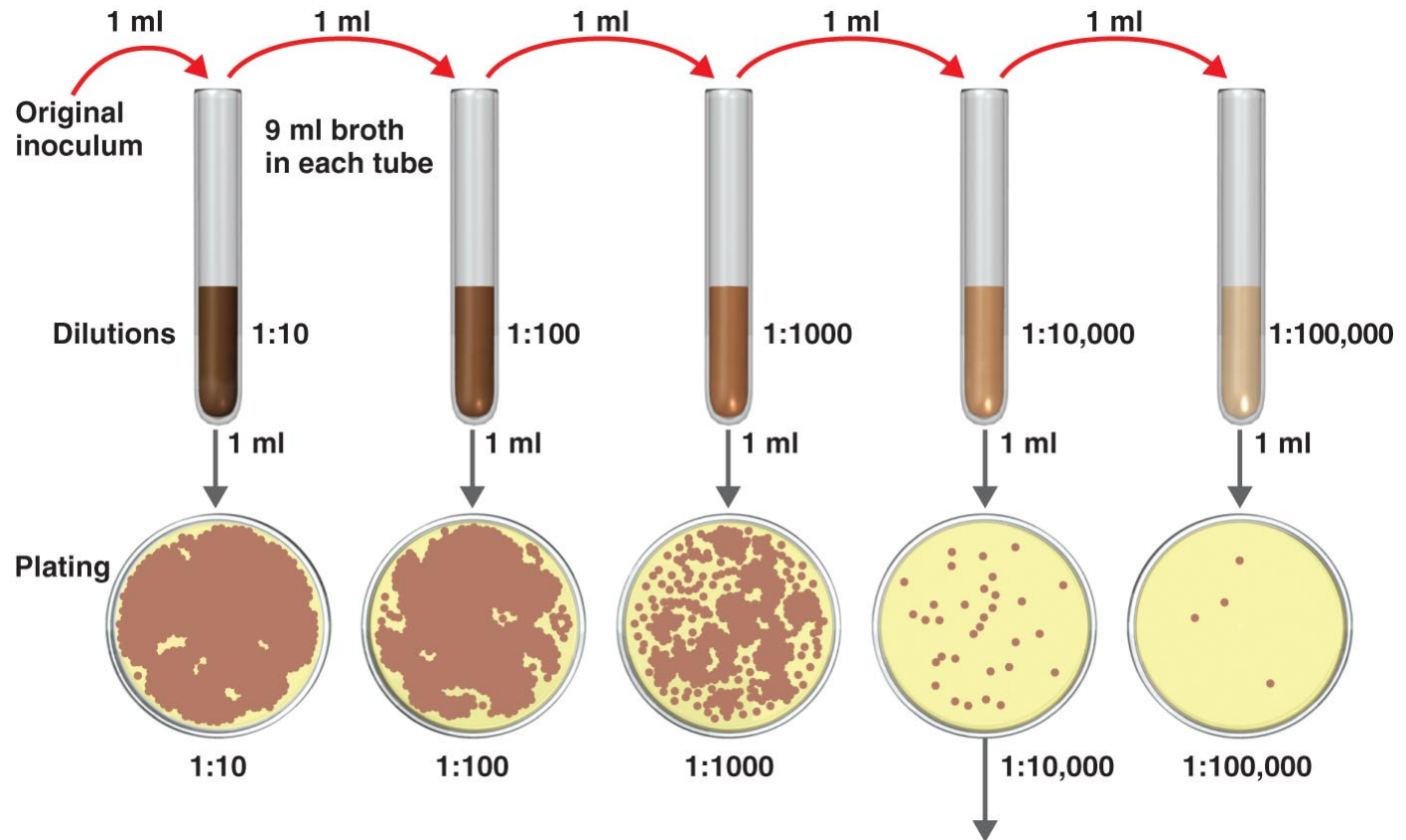


Fig. 6.17

## II. Exp. 6: Standard Plate Count



Calculation: Number of colonies on plate  $\times$  reciprocal of dilution of sample = number of bacteria/ml  
(For example, if 32 colonies are on a plate of 1:10,000 dilution, then the count is  $32 \times 10,000 = 320,000$  bacteria/ml in sample.)

Fig. 6.16

## **II. Exp. 6: Standard Plate Count**

- ▶ **A. Sponge Water or Rinsed Bagged Lettuce**
  - Work with a partner
  - **Whenever testing a liquid, test the undiluted sample or  $10^0$  dilution**
  - Use 9 ml sterile  $H_2O$  test tubes
  - Prepare plates from  $10^0$ – $10^{-6}$
  - Refer to Figure 5 in the Lab Manual
  - To be demonstrated by instructor

## II. Exp. 6: Standard Plate Count

- ▶ **B. Ground Raw Turkey Meat (more lean)**
  - Work with a partner
  - **Whenever testing a solid, you must test a diluted specimen first ( $10^{-1}$ )**
  - Your instructor will prepare the ground turkey sample and provide you with a  $10^{-1}$  dilution sample that you will begin testing
  - Use 99 ml sterile  $H_2O$  bottles
  - Prepare plates from  $10^{-1}$ – $10^{-6}$
  - Refer to Figure 6 in the Lab Manual
  - To be demonstrated by instructor

