BIO 201 Lab 4
Experiment 5 & 6

Professor Diane Hilker

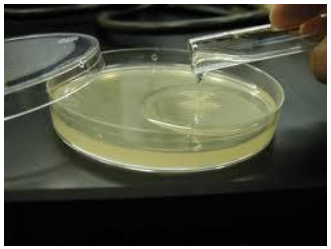
Overview

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.
- **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 100ºC/Hardens at 42ºC
  - 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 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

- Standard Pour Plate Method

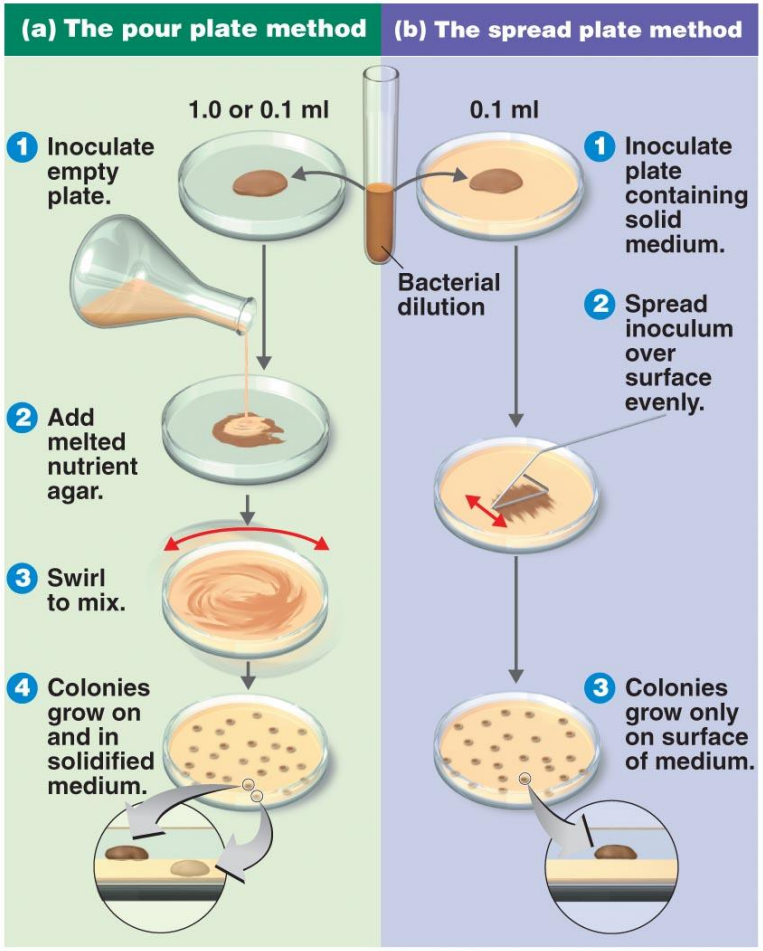
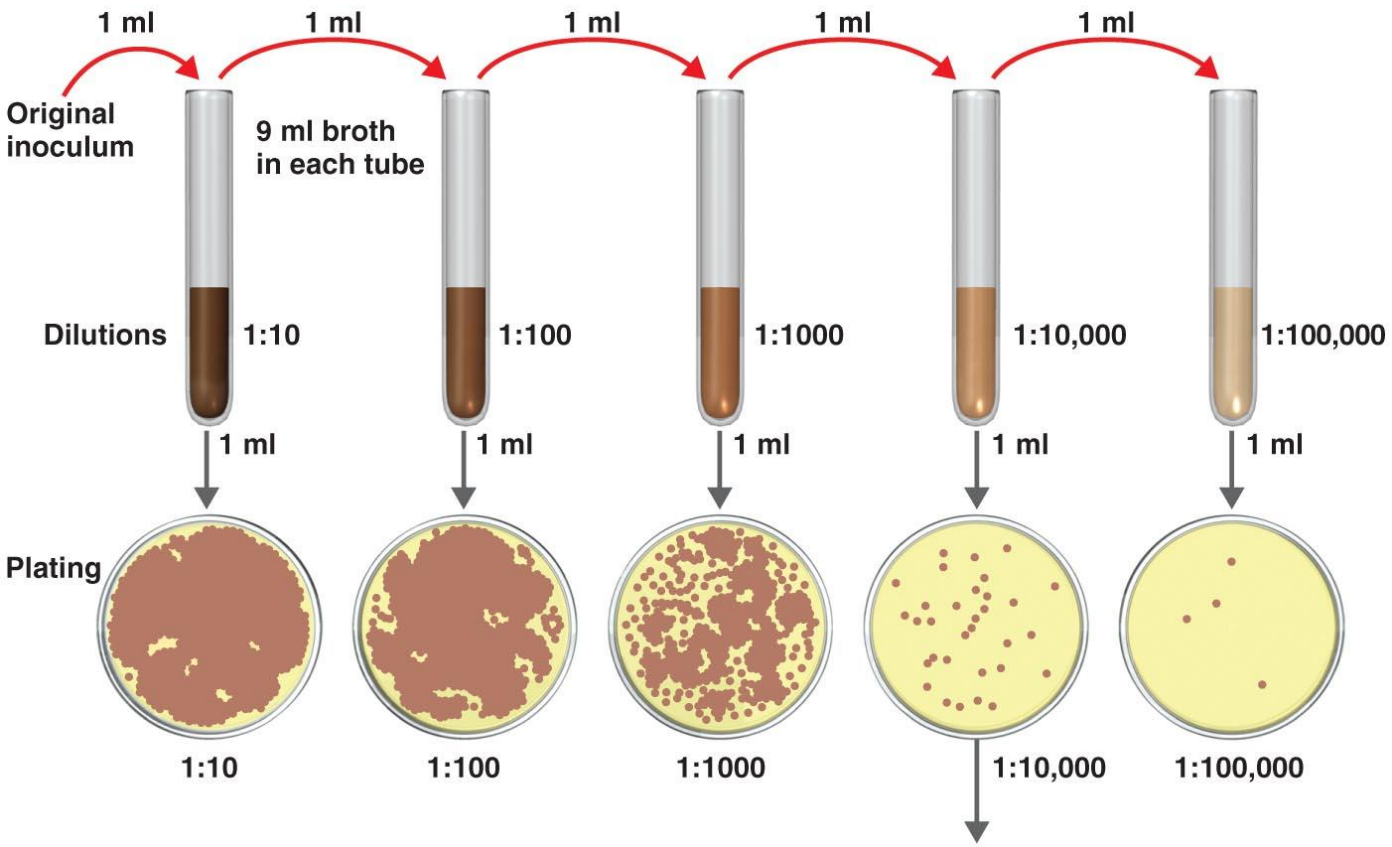


Fig. 6.17

II. Exp. 6: Standard Plate Count



Calculation: Number of colonies on plate × 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 × 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}
  - See Figure 5 in 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})**
  - Use 99 ml sterile H_2O bottles
  - Prepare plates from 10^{-1}–10^{-6}
  - See Figure 6 in Lab Manual
  - To be demonstrated by instructor