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 × 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.)
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$_2$O 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\(_2\)O bottles
   ◦ Prepare plates from \(10^{-1}–10^{-6}\)
   ◦ Refer to Figure 6 in the Lab Manual
   ◦ To be demonstrated by instructor