

## General Microbiology Laboratory

### Lab Session 11 - Quantification and Characterization of Microorganisms in Food

#### Introduction

Microorganisms have several types of relationships with food. Microorganisms are used to produce food (largely due to the prolific fermentative metabolism of specific bacteria), they can initiate the processes involved in food spoilage (largely due to their prolific reproduction and the role of specific bacteria as decomposers), and they can be a source of human disease (if food is contaminated with microorganisms that can infect humans or if they produce toxins that can be ingested). Unfortunately, infectious or toxic doses of microorganisms can be present in foods before the food is recognizable as spoiled. Foods can contain  $10^5$  to  $10^6$  mo's per gram without being detected. The purpose of this lab is to determine the approximate number and diversity of microorganisms in various food substances.

#### A. Review the following for reference:

1. Lab Manual: Exercises Exercise #53 - Pages 321 to 324.
2. Text: Chapter 40

#### B. Do the following during lab:

##### Preparation of Food Sample:

1. Use a spatula and weigh out a one gram sample of food. OR, Use a 1 ml pipet to collect 1 ml of liquid.
2. Transfer the food sample to a 9 ml water blank. This is a 1:10 dilution.  
Note: It will be necessary to homogenize or finely slice meats or other solids.
3. Cap the tube tightly and shake it until your arm becomes weak. Allow the solids to settle and the lipids to float.  
Note: Use the aqueous phase to inoculate culture media.

##### Inoculation of Culture Media:

1. Inoculate the following media with 100 ul (this will be another 1:10 dilution) of the prepared food sample:

<u>Culture Media</u>	<u>Purpose</u>
Trypticase Soy Agar	Total number of microorganisms in food
Columbia Agar	Total number of gram positive organisms.
Mannitol Salt Agar	Isolation and identification of staphylococci including <i>S. aureus</i> .
MacConkey Agar	Total number of gram negative microorganisms
Eosin-Methylene Blue Agar	Isolation and identification of coliforms, especially <i>E. coli</i> .
Hektoen Enteric Agar	Isolation and tentative identification of various gram negative mo's including <i>Salmonella sp.</i> , <i>Shigella sp</i> and <i>Proteus sp</i> .

2. Spread the inoculum over the surface of the media with a swab. One swab will work for all plates.
3. Incubate at 37 C for 24-48 hours.
4. Evaluate the microbial growth that occurs on each of the plates.

#### C. Techniques:

##### Quantitative analysis of microorganisms in raw food

1. Weigh out 1 gram of food (e.g. hamburger) and place in a blender with 9 ml of 0.9% sterile saline or distilled water. Blend for 3 minutes on high speed.
2. Do 2 to 4, 10-fold dilutions of the supernate..
3. Plate 100 ul of the dilutions on the appropriate culture media.
4. Count colonies to determine the exact number of mo's per gram of food.  
Colonies counted x dilution factor = # of mo's per gram of food.

##### Qualitative analysis of microorganisms in raw food

1. Isolate colonies of suspected pathogens
2. Identify using appropriate biochemical tests.

#### D. Results

1. Estimate the number and type of microorganisms in the prepared food sample:
  - a. Number of colonies: Colonies counted x 100 (the dilution factor) = # of mo's per gram of food.
  - b. Presumptive identification based on growth and appearance on selective/differential media.  
For example: *Staph.sp.*, *E. coli*, *Salmonella sp.*, *Shigella sp* and *Proteus sp*.
2. Comment on the microbial content of the food, as well as tap water (done as a Demonstration).

Food & Source: \_\_\_\_\_

Culture Media	Estimated MO/gr	Presumptive Identification	Comments
Trypticase Soy Agar			

Columbia Agar

Mannitol Salt Agar

Eosin-Methylene Blue Agar

Hektoen Enteric Agar

Analysis of Water:

## **E. Discussion:**