

## **Microbial Examination of Food**

Our food is increasingly produced in large, commercial facilities. Economics has driven the shift of animal production from small farms to huge, densely packed feedlots for cattle and sheep and enclosed buildings for chicken and pigs. Antibiotics are routinely used to control outbreaks of diseases common under these conditions. These animals are processed in plants in mind-boggling numbers. The sheer volume of animals being processed makes maintaining “clean” conditions difficult.

In 1906, Upton Sinclair's novel “The Jungle” exposed the deplorable conditions in the nation's meatpacking plants, prompting the passage of the nation's first food inspection laws. Now any facility that processes, sells, or serves food must prominently post their inspection rating from the health department. The numerous outbreaks of microbial caused food borne diseases have created an increasing demand for vigilance in maintaining the safety of our food supply. Newer methods, such as irradiation, for ensuring “clean” food have created new controversy about the possible health consequences of these procedures. Please remember that food is required by the Food and Drug Administration just to have less than a certain level of bacterial contamination; not be microbial free. Our bodies are uniquely designed to handle a “normal” level of microbial contamination.

### **Overview**

You first need to get the bacteria out of or off of the sample. Because we have a limited number of blenders available you will blend, shake or dilute your food sample. You will blend/shake your sample up with sterile water, dispense a small amount of the blended fluid onto a Plate Count Agar (PCA), allow the bacteria to grow for 48 hours, and then count the number of colonies that grow. We are following a slightly modified version of the standard the American Public Health Association (APHA) microbial contamination protocol or standard method. In the original protocol, the diluted food sample is added to molten and slightly cooled agar and then plated. This is difficult to do without each table having their own water bath. Dispensing the sample directly onto the agar surface is an acceptable alternate method.

Since you don't know how much bacteria is in your sample, you will plate several dilutions. Hopefully one of the plates will have between 30-300 colony forming units (CFU's). The information you obtain about the microbial contamination reflects only those organisms that can grow on Plate Count Agar (PCA) at the incubation temperature and in the incubation time of the test conditions. It would be useful to review the section on determining population counts using serial dilutions. PCA is a very clear agar that allows you to easily see the colonies.

### **Procedure:**

The food samples will be provided for you by the prep lab. Your lab dollars at work for you! Remember to use sterile technique to avoid contaminating the sample; this could result in plates with higher colony forming units (CFU's). Remember that you should have a minimum of three samples for each dilution level for statistical analysis.

### **Day 1:**

Follow the procedure appropriate for your food sample.

#### 1. Solid Food...

Using a sterile spatula and sterile petri dish, weigh out 11 g of sample. Add the sample to a sterile blender filled with one bottle of the sterile water, 99 ml. Blend for several minutes to disperse the bacteria. This is a 1:10 dilution.

OR

Using clean gloves, weigh out 11g of sample and put it into a wide-mouthed sterile 99mL water blank. Shake the bottle in an arc 20 times with your elbow resting on the table. Be sure you have the lid on tight!

Liquid Food (i.e. milk, yogurt, etc)...

Pipette 11mL into a 99mL water blank. This is a 1:10 dilution. Shake the bottle in an arc 20 times with your elbow resting on the table. Be sure you have the lid on tight!

2. Add 1mL of this solution to another sterile 99mL water blank. Mix well using the standard bottle shaking technique. (It is actually part of the APHA protocol). Label this dilution bottle, 1:1000 or 1:10<sup>2</sup>.
3. Add 1mL of your 1:10<sup>2</sup> dilution to another sterile 99 ml water blank. Mix well using the standard bottle shaking technique. (It is actually part of the FDA protocol). Label this dilution bottle, 1:100000 or 1:10<sup>4</sup>.
4. Label the bottom of your petri plates 1:10, 1:10<sup>2</sup>, 1:10<sup>3</sup>, 1:10<sup>4</sup>, 1:10<sup>5</sup>
5. Add 1mL from your 1:10 dilution, the blender, or first shaker or dilution bottle to a 1:10 plate. Smear the sample evenly across the plate with a hockey stick. This solution may be difficult to pipette, especially if your sample is hamburger. You will probably need to use a 5mL pipette. And yes, it may be yucky!
6. Add 1mL of your 1:1000 dilution to the labeled 1:10<sup>2</sup> plate and smear. Be sure to accurately label the bottom of each plate with the sample you put on it, your group and the class.
7. Add 0.1mL of your 1:1,000 dilution to the labeled 1:10<sup>3</sup> plate and smear.
8. Add 1mL of your 1:100,000 dilution to the labeled 1:10<sup>4</sup> plate and smear.
9. Add 0.1mL of your 1:1,000 dilution to the labeled 1:10<sup>5</sup> plate and smear.
10. Let the plates sit on the counter until all the liquid is absorbed into the agar surface.
11. Incubate the plates upside down for 48 hrs at 35°C

## DAY 2

1. Arrange the plates on your counter from lowest to highest concentration.
2. Select the plate that has between 30 and 300 colonies. Counting less than 30 colonies does not give statistically reliable results. Counting more than 300 colonies is exceedingly difficult. If your most dilute plate (1:10<sup>5</sup>) has more than 300 colonies, you can estimate the number of colonies by using a grid available in lab, counting 10 squares and multiplying that value by 7.4 to give you an approximate CFU/plate.
3. Use the following equation to calculate the CFU/gram or ml of the original sample.

$$\frac{\text{CFU/gram or CFU/mL}}{\text{CFU/mL}} = \frac{\text{CFU Number}}{\text{Volume Plated (mL) X Dilution Factor}}$$