**Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

**SERIAL DILUTIONS & MILK MICROBIOLOGY**

**Part I: Serial Dilution**

Quantifying bacteria can be a difficult task to achieve using direct methods of enumeration. Cell-counting instruments exist that can be used to count numbers of organisms in a sample using electrical or light impedance, but these tools are often not found in every lab setting. The viable cell count is an estimate of bacterial population in an original sample being tested. To perform viable cell counts on agar plates, it is often necessary to dilute the original sample to make counting easier. Countable plates are typically considered to hold 30-300 colonies on the surface of the agar. This lab teaches the serial dilution method as well as the spread plate method used to perform a viable cell count.

**Materials:**

* Pure Culture of *Micrococcus lutea*
* Samples of different milk types, dates, etc.
* Eight 9-ml water blanks per team  
  Eight agar plates per team  
  Ten sterile disposable 1ml pipettes per team  
  Spreading rods
* Colony counter available for next (evaluation) lab

**Procedure:**

In groups of 2-4, complete the serial dilutions of a known specimen as diagramed by the instructor on the board.

 One milliliter of the original sample is transferred to a tube containing 9ml of sterile water. This tube now contains 10 total ml of solution, 1 part sample and 9 parts water. We call this tube a 1 in 10 dilution. If one milliliter of solution is moved from this tube to another tube with 9ml of sterile water, then we have created a 1:100 dilution. This process is carried out until desired dilution factors are met. After the proper dilutions are met, then the contents of each tube can be used to create plates. Typically, we have students use ½ ml of the diluted samples from each tube on a plate of its own. This renders plates with dilution one half of the original dilution, meaning that a plate from the 1:10 dilution is actually a 1:20 dilution plate.

*[NOTE: Students often confuse the numbers on the pipette.  The entire pipette filled to the 0 contains only one ml.  To inoculate with ½ ml, let the pipette empty down to the .5 ml mark (half the contents).  You may want to fill the pipette only halfway full in the first place].*

**Serial Dilution of a Known Organism**

Each group will perform a serial dilution using a known organism, *Micrococcus lutea*. This organism produces small yellow colonies on the agar surface making it fairly easy to count.

1.       Using a pure culture of *M. lutea*, transfer 1ml of organism from the culture tube to the first sterile water blank. Label this tube 1:10.

2.       Mix the tube contents using the vortex mixer on the end of each table.

3.       Using another transfer pipette, transfer 1ml of the 1:10 tube to the next sterile blank tube. Label this tube 1:100.

4.       Continue making transfers until 1:100 and 1:10000 dilutions have been made.

5.       After all dilution have been completed, transfer ½ ml from the 1:10 dilution and place it on a plate labeled 1:20.

6.       Spread the liquid across the surface of the plate with a clean spreading rod.

7.       Continue making plates in this fashion from each of the dilution tubes until you have created four plates: 1:20, 1:200, 1:2000, 1:20000.

8.       Place the inverted plates in a rack to be incubated.

**Serial Dilution of Milk Samples**

Each group will choose a milk sample and use the techniques learned to perform a set of serial dilutions and spread plates for the sample.

1.       Using a sample of milk, transfer 1ml into the first sterile water blank. Label this tube 1:10.

2.       Mix the tube contents using the vortex mixer on the end of each table.

3.       Using another transfer pipette, transfer 1ml of the 1:10 tube to the next sterile blank tube. Label this tube 1:100.

4.       Continue making transfers until 1:100 and 1:10000 dilutions have been made.

5.       After all dilution have been completed, transfer ½ ml from the 1:10 dilution and place it on a plate labeled 1:20.

6.       Spread the liquid across the surface of the plate with a clean spreading rod.

7.       Continue making plates in this fashion from each of the dilution tubes until you have created four plates: 1:20, 1:200, 1:2000, 1:20000.

8.       Place the inverted plates in a rack to be incubated.

**Spread Plate Counts**

Students should count the known organism plates and the milk plates to determine the number of colonies. Then a calculation of the viable cells in the original sample can be performed. A colony is the result of rapid division of one or a few cells giving rise to a visible mass. This allows you to estimate the number of cells or colony forming units (CFUs) in the original sample by counting the number of colonies and multiplying by the dilution factor of the plate.

**Example:  If the 1:200 plate shows 148 living colonies**

**148 x 200=29,600 CFUs in 1ml of the original sample**

*[Any plate containing less than 300 colonies but more than 30 should be counted.  Plates containing fewer than 30 colonies are considered statistically insignificant and subject to sampling error.  Plates containing over 300 colonies are overcrowded and colonies are likely to merge in growth, preventing accuracy in counting.]*

Realize that this test doesn’t provide for anaerobic growth, nor are the conditions ideal for psychrophillic or thermophilic growth.  However, a useful indication of degree of contamination is obtained. The lighted counting grid may help.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *M. Lutea* | Colonies counted | CFUs/ ml | Milk | Colonies counted | CFUs/ ml |
| 1:20 |  |  | 1:20 |  |  |
| 1:200 |  |  | 1:200 |  |  |
| 1:2000 |  |  | 1:2000 |  |  |
| 1:20000 |  |  | 1:20000 |  |  |

Milk is normally sterile as it forms in the udder of the cow.  **Contamination** occurs as the milk is passed through the ducts of the udder.  *Lactobacillus* and *Streptococcus* species become milk contaminants at this point.  These are normal in raw or pasteurized milk and rarely would be involved in **pathogenesis**.  They do, however, contribute to **souring** of milk.  Cool temperatures prolong the **palatability** of such milk.  Additional contamination can occur from dust, manure, polluted water, poor processing techniques such as unclean mechanical equipment and unsanitary handling of the dairy products by personnel.  Common contaminants are *Pseudomonas*, *Clostridium*, *Flavobacterium*, *Bacillus*, and *Micrococcus*.  If the cow is infected with a pathogenic organism such as *Mycobacterium sp*., the milk provides a means of transmission to humans.

**Pasteurization** involves heating the milk to temperatures, which would eliminate **fastidious** pathogens, but not alter the food value and palatability of the milk.  Such heating does not sterilize the milk, as the *Lactobacillus* will survive the temperature.  However, the bacterial count is greatly reduced and the pathogens are killed.

For Grade A Government (USDA) inspected milk, pasteurized milk should not contain over 20,000 bacteria/ml.  Quality raw milk for distribution or subsequent pasteurization may not contain over 200,000 bacteria/ml.  **Coliform bacteria** may not exceed 10/ml.