# Food Product Sampling and Standard Plating Methods

## Introduction

### Food Sampling:

Food product sampling is the process of taking raw or ready to eat (RTE) food product and manipulating it into a form that can be distributed into microbiological media or used for further chemical or biological tests. The methods used for sampling food will differ depending on the characteristics of the food and the types of analyses being performed. In general, liquid foods can be diluted into sterile liquid medium, and solid foods can be physically altered through mixing, cutting, blending, or stomaching to produce a liquid homogenate. Both types of food can then be further diluted and distributed into enrichment media, microbiological agar plates, or used for other laboratory tests.

### Standard Plate Counts:

The Standard Plate Count (SPC) is the plating technique used to estimate the amount of aerobic bacteria present in a particular sample. It is sometimes referred to as the Aerobic Plate Count (APC) or Total Plate Count (TPC). These methods utilize a media mixture of non-selective nutrients necessary for bacterial growth which are suspended in a pectin gel. Using the pour plate method, liquid samples are mixed in a Petri dish with the molten growth media and allowed to solidify. In the spread plate method, liquid samples are distributed and spread evenly onto the pre-poured solidified agar surface. Incubation of these methods is performed under the same conditions. Petrifilm is another type of media that uses the same methods as SPC. It utilizes a film of nutrient media containing a gelling agent that is activated once liquid samples are properly distributed onto the film surface. The Aerobic Count Petrifilm can also be used to determine standard plate counts.

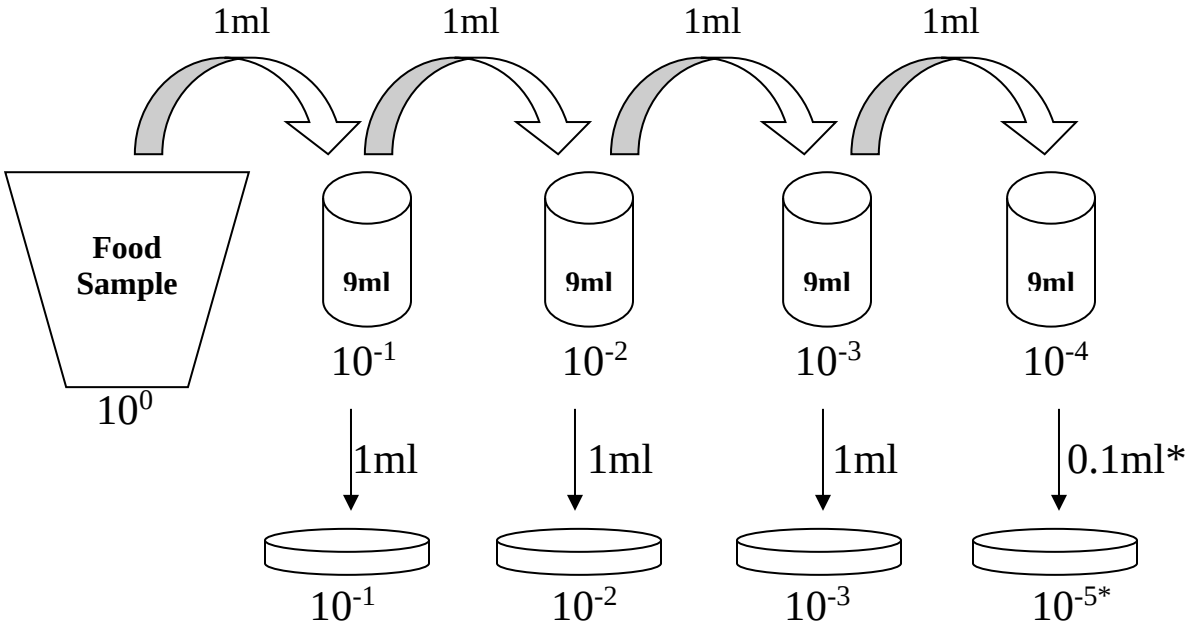
### Spiral Plating:

The spiral plating method uses a mechanical liquid dispenser that distributes a set volume of liquid onto the surface of a rotating, pre-poured agar Petri dish. As the sample is distributed away from the starting position, smaller amounts are deposited onto the surface of the agar in a spiral motion, thus achieving a dilution effect. Because the volume of each segment of the plate is known, multiple dilutions of the same sample can be enumerated on the same plate. Following incubation, colonies can be enumerated manually or by computerized colony counters. This method is highly reproducible, uses fewer supplies, and requires minimal training of personnel.

**Dilutions:**

When sampling a food product with an unknown concentration of bacteria, it is necessary to dilute the homogenized sample in order to determine the estimated bacterial concentration. Since bacteria divide and grow in an exponential fashion, a logarithmic scale to estimate the concentration of bacteria in a food is typically used. Based on this concept, a series of 10-fold dilutions can be performed on the homogenized sample, the sample plated, and the resulting colony count converted into a logarithmic form.

**A Typical Dilution Scheme**




*Altering the volume of sample pipetted changes the dilution factor. Transferring 0.1ml increases the dilution factor one fold. In this scheme, pipetting 0.1ml results in a $10^{-5}$ dilution whereas transferring 1ml would have resulted in a $10^{-4}$ dilution.

**Enumeration:**

Once a food product has been sampled, plated, and incubated, the resulting bacterial growth is enumerated, or counted. Bacterial colonies will appear in a variety of shapes and colors depending on the type of agar used, the genus and species of bacteria grown, the incubation conditions, and presence of growth inhibitors or promoters. Although one colony could contain more than a million cells, each colony is representative of one originating bacterial cell and is called a colony forming unit (CFU). Once a raw colony count has been completed, the dilution factor and the quantity of sample are incorporated into the count, and a logarithmic value is calculated. Typically, standard counting

methodologies dictate that only plates containing between (25-250 colonies) should be counted, and counts outside that range should be clearly labeled as estimated counts.

**Example Counting Exercise**

| Dilution | Raw CFU | Average CFU | Scientific Notation | CFU/ml |
|---|---|---|---|---|
| $10^{-2}$ | 100,124 | 112 | $112 \times 10^{2}$ | $1.12 \times 10^{4}$ CFU/ml |
| $10^{-3}$ | 25, 27 | 26 | $26 \times 10^{3}$ | $2.6 \times 10^{4}$ CFU/ml |
| $10^{-4}$ | 6, 4 | 5 | $5.0 \times 10^{4}$ | $5.0 \times 10^{4}$ CFU/ml est. |

| CFU/ml | Converted to Logarithmic Form |
|---|---|
| $1.12 \times 10^{4}$ CFU/ml | 4.05 log CFU/ml |
| $2.6 \times 10^{4}$ CFU/ml | 4.4 log CFU/ml |
| $5.0 \times 10^{4}$ CFU/ml | 4.7 log CFU/ml est. |

## Protocol

Materials Needed:

1. Food products to sample
2. Scale
3. Stomacher
4. Stomacher bags
5. Sterile spoons and knives
6. Sterile buffered peptone water (BPW) for stomaching
7. Pipettors
8. 10 ml pipettes
9. 1 ml pipette tips
10. 9 ml dilution blanks
11. 99 ml dilution blanks
12. 6 empty petri dishes for pour plating
13. 6 pre-poured PCA plates for spread plating
14. 6 tubes of 15 ml molten plate count agar
15. 6 APC Petrifilm
16. 6 *E.coli*/coliform Petrifilm
17. 6 Yeast/Mold Petrifilm
18. Petrifilm spreaders
19. Disposable plastic bent spreaders

## Food Samples and Plating Categories:

| Food Item | Plating Method |
|---|---|
| Ground Beef | Pour Plate |
| Milk | Spread Plate |
| Dry Spice | Petrifilm |

## Food sample preparation

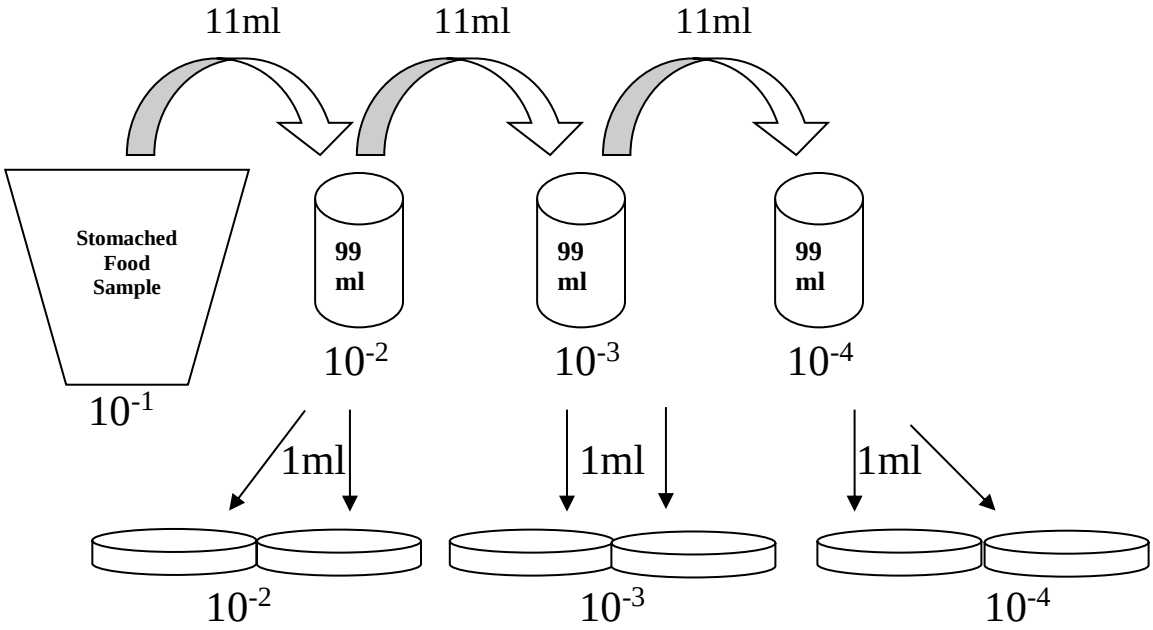
Solid food (ground beef and dry spice):

1. Place a new sterile stomacher bag into the beaker sitting on the scale and "zero" the scale.
2. Using a sterile spoon or forceps, remove small amounts of food sample from its packaging and place into the stomacher bag until the scale reads 10 grams. (Remember to sanitize the outside of the food package, if possible, with an alcohol pad before removing the food sample).
3. Slowly pour sterile BPW into the stomacher bag until the scale reads 100 grams, to make your initial 1:10 ($10^{-1}$) dilution.
4. Stomach sample for 90 seconds.
5. Bring stomached sample back to your lab bench and prepare for dilutions and plating.

Liquid food (milk):

1. Obtain the milk sample and a 9 ml dilution blank and return to your lab bench.
2. Pipette 1 ml of the milk sample into the 9 ml dilution blank.
3. Shake the dilution blank 25 times in an arc of 30 cm (1ft) within 7 seconds.
4. You have now produced a ($10^{-1}$) dilution, label bottle accordingly.
5. If dilution bottle sits for more than 3 min, re-shake before plating.
6. Prepare for dilutions and plating.

## Ground Beef – Pour Plate

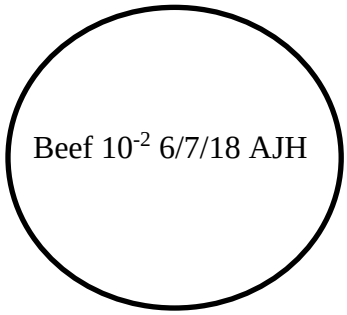



Preparation:

1. Label 99 ml dilution blanks with the dilution value ($10^{-2}$, $10^{-3}$,$10^{-4}$).
2. To produce 10-fold serial dilutions, pipette 11 ml from the homogenized stomachate into the first 99 ml dilution blank.
3. Vigorously shake the dilution blank 25 times in an arc of 30 cm (1ft) within 7 seconds.
4. Pipette 11 ml from the shaken dilution blank into the next 99 ml dilution blank, and repeat until all dilutions have been completed.

Pour Plate:

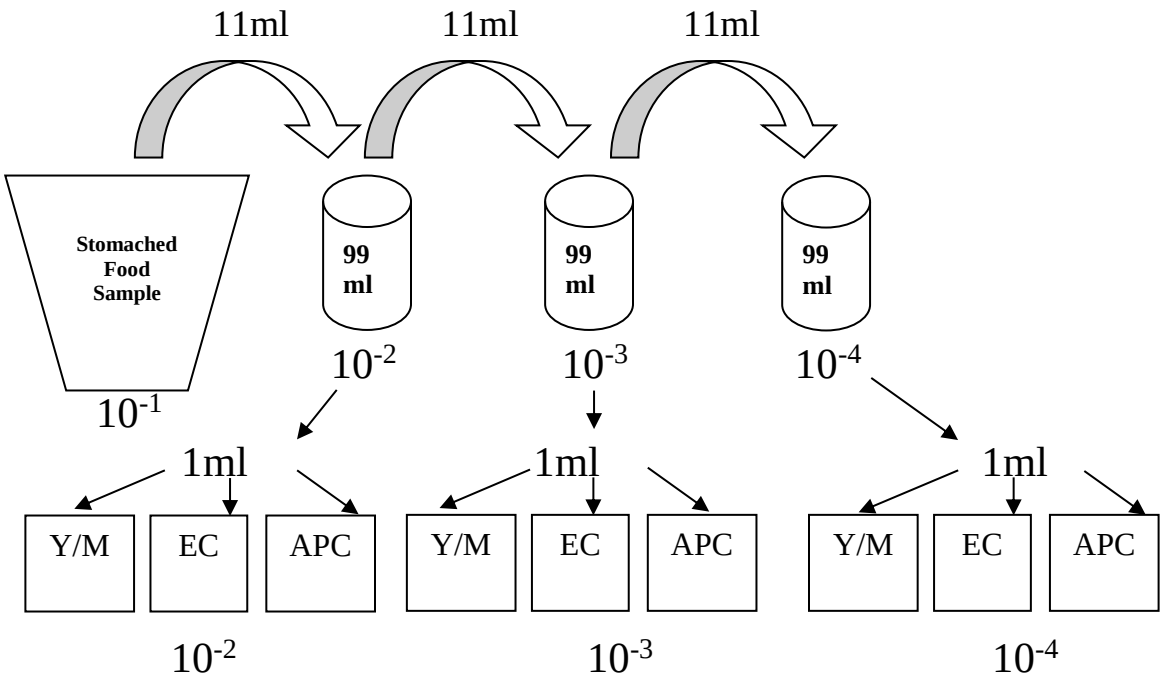
1. Label empty Petri dishes with the correct dilution, in duplicate, with your initials and date (i.e. $10^2$ 6/1/13 JDA).




2. Pipette 1 ml of each prepared dilution, in duplicate, into an empty Petri dish.

3. Pour the 15 ml of molten PCA agar onto the 1 ml sample contained in the Petri dish.
4. Gently swirl the closed Petri dish in a "figure eight" motion to thoroughly mix the sample. Do not splash agar on the lid.
5. Set the Petri dishes aside for 15 minutes and allow to harden.
6. Once the agar has hardened, flip the Petri dishes upside down and incubate at 35°C for 24 hours.

**Dry Spice – Petrifilm®:**



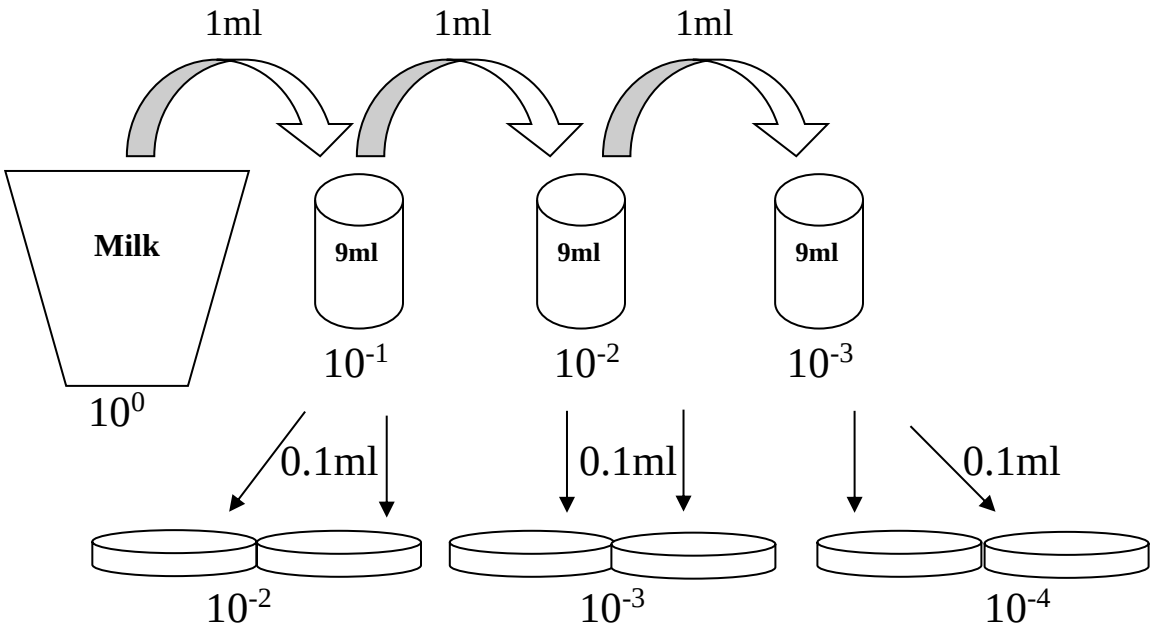

Preparation:

1. Label 99 ml dilution blanks with the dilution value ($10^{-2}$, $10^{-3}$,$10^{-4}$).
2. To produce 10-fold serial dilutions, pipette 11 ml from the homogenized stomachate into the first 99 ml dilution blank.
3. Vigorously shake the dilution blank 25 times in an arc of 30 cm (1ft) within 7 seconds.
4. Pipette 11 ml from the shaken dilution blank into the next 99 ml dilution blank, and repeat until all dilutions have been completed.

Petrifilm Plating:

1. Label Petrifilm cards (APC, YM, EC) with the correct dilution with the food sample, dilution, your initials and date.
2. Pipette 1 ml of food sample onto each Petrifilm by following these instructions:
    a. Lift the outer Petrifilm cover.
    b. Slowly transfer 1 ml of sample onto the middle of the exposed film surface.
    c. Slowly place the outer cover back down to its original position, avoiding bubbles.
    d. Gently place the appropriate spreader onto the outer cover of the Petrifilm and **gently** push on the spreader until all of the sample is evenly spread across the spreader. The sample should form a circle in the Petrifilm surface.
    e. Remove the spreader and allow the Petrifilm to sit for 5 min.
    f. Stack the Petrifilm on top of each other and incubate at 35°C for 24 hours (Incubate Yeast/Mold at 25°C for 1-3 days).

**Milk:**




Preparation:

1. Label dilution blanks with the dilution value, remember the mixed liquid sample is already at a ($10^{-1}$) dilution, so your next dilutions will be ($10^{-2}$, $10^{-3}$), if transferring 1 ml into 9 ml dilution blanks.
2. To produce 10-fold serial dilutions, pipette 1 ml from the milk and buffer mixture into the first 9 ml dilution blank.
3. Vigorously shake the dilution blank 25 times in the arc of 30 cm (1ft) within 7 seconds.
4. Pipette 1 ml from the shaken dilution blank into the next 9 ml dilution blank, and repeat until all dilutions have been completed.

Spread Plating:

1. Label pre-poured agar plates with the correct dilution, in duplicate, with your initials and date (i.e. $10^2$ 6/1/13 JDA).
2. Pipette 0.1 ml (100 microliters) of each prepared dilution, in duplicate, onto the pre-prepared agar plates. *(Remember that when you plate 0.1 ml you are increasing the dilution one fold).*
3. Using a sterile plastic spreader, spread the sample evenly across the plate in all directions, spinning the plate if necessary.
4. Allow the plates to sit for 1 minute, then flip the Petri dishes upside down and incubate at 35°C for 24 hours.