



FOOD SAFETY LAB / MILK QUALITY  
IMPROVEMENT PROGRAM  
*Standard Operating Procedure*



Title: **Preparing and spiral plating fluid and powdered dairy products**

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## **SECTION 1 INTRODUCTION**

### **1.1 Purpose**

The purpose of this document is to set forth standard guidelines for spiral plate counts of raw, pasteurized, and powdered dairy products.

### **1.2 Scope**

This SOP applies to the Food Safety Lab and the Milk Quality Improvement Program. The protocols may also be used by laboratory members from other locations.

### **1.3 Definitions**

SPC- Standard Plate Count agar

BHI- Brain Heart Infusion agar

### **1.4 Safety**

Wear gloves, safety glasses, and other appropriate personal protective equipment for the entire procedure.



## SECTION 2 MATERIALS

- **Standard Plate Count (SPC) Agar.** Pre-poured SPC plates must be prepared and dried prior to use with the spiral plater.
- **Brain Heart Infusion (BHI) Agar.** Pre-poured BHI plates must be prepared and dried prior to use with the spiral plater.
- **70% Ethanol**
- **Kimwipes**
- **Incubators** Set at 55°C and 32°C
- **Spiral Plater**
- **Phosphate Buffer Dilution Blanks.** 900 µL and 99 mL volumes.
- **Variable Volume Pipettes.** 100-1000 µL volume and corresponding tips
- **Vortex**



## SECTION 3 PROCEDURES

### 3.1. Pre-poured agar plate preparation

3.1.1. SPC and BHI agar plates (or other appropriate media) are prepared using an automatic dispenser with sterile delivery system. The plates should solidify on a level surface then can be stored in closed bags at 4°C prior to use. Prior to plating plates should dry and come to room temperature.

### 3.2. Sample preparation

#### 3.2.1. Fluid milk sample preparation

3.2.1.1. Fluid milk samples are maintained at or below 6°C from arrival to completion of analysis. Analysis begins within 24 hours of sample receipt.

3.2.1.2. Samples are shaken 25 times in a 1-foot arc within 7 seconds in accordance with *Standard Methods for the Examination of Dairy Products* (Laird et al., 2004) prior to plating, dilution, or heat treatment. If the sample is not plated or diluted within 3 minutes re-shake sample.

3.2.1.3. Prior to opening the container, wipe the container down with a Kimwipe and 70% ethyl alcohol.

3.2.1.4. Raw milk can now be plated, or heat treated.

#### 3.2.2. Powdered product sample preparation

3.2.2.1. Powdered products are aseptically mixed to ensure a uniform sample.

3.2.2.2. 11g of powdered product is transferred directly into 99 mL of phosphate buffer (1:10 dilution).

3.2.2.3. Hydrated powder products are shaken 25 times in a 1-foot arc within 7 seconds prior to plating or heat treatment. If the sample is not plated within 3 minutes re-shake sample.

3.2.2.4. Powdered product sample can now be plated, or heat treated.

### 3.3. Sample plating

#### 3.3.1. Plating of non-heat-treated samples

3.3.1.1. Label 4 pre-poured SPC plates per non-heat treated raw or powder product with product identifier, dilution, and date.

3.3.1.2. Each non-heat-treated product is plated undiluted as well as at 1:100 dilution, both in duplicate. Other dilutions may be done depending on the expected bacterial concentration.

3.3.1.3. Plate each sample using the 50 Exponential mode on the spiral plater, cleaning, or changing tips, between each sample.



- 3.3.1.3.1. Follow the “Autoplate 5000 Spiral Plater and QCount” and “Eddy Jet 2 Spiral Plater and Sphere Flash Colony Counter” SOPs located on the wiki for spiral plater use and for enumeration
- 3.3.1.4. Incubate plates at 32°C for 24-48 hours prior to performing colony counts.
- 3.3.2. Plating of heat-treated samples
  - 3.3.2.1. Label enough BHI plates to plate undiluted heat-treated sample in duplicate.
    - 3.3.2.1.1. The sensitivity of this test can be increased by plating in quadruplicate as opposed to in duplicate.



**SECTION 4**

**TROUBLESHOOTING**

**SECTION 5**

**REFERENCES**

*Laird, D. T., A. Gambrel-Lenarz, F. M. Scher, T. E. Graham, and R. Reddy. 2004. Microbiological Methods for Dairy Products. Pages 249–252 in Standard Methods for the Examination of Dairy Products. 17<sup>th</sup> ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.*

*Maturin LJ, Peeler JT. 2001. Aerobic plate count. In: Bacteriological analytical manual online, 8th edn. Center for Food Safety & Applied Nutrition, US Food and Drug Administration. Available online [<http://www.cfsan.fda.gov/~ebam/bam-3.html>]*

*Wehr, H. M. and J. F. Frank eds. 2004. Standard Methods for the Examination of Dairy Products. 17th ed. American Public Health Association, Washington, DC.*

Autoplate 5000 Spiral Plater and Q-Count SOP

Eddy Jet 2 Spiral Plater and Sphere Flash Colony Counter SOP

**SECTION 6**

**METHOD VERSION & CHANGES**

| <b>VERSION</b> | <b>DATE</b> | <b>EDITOR</b>    | <b>COMMENTS</b>   |
|----------------|-------------|------------------|---|
| Version 1      | 11/21/2011  | Nicole Martin    | Original SOP  |
| Version 2      | 4/16/2020   | Rachel Evanowski | Updated to new formatting and fixed grammatical errors. Changed title from “Spiral plating for raw and powdered dairy products” |
| Version 3      |             |                  |   |