



FOOD SAFETY LAB / MILK QUALITY
IMPROVEMENT PROGRAM
Standard Operating Procedure



Title: **Highly Heat Resistant Spore Pasteurization**

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Highly Heat Resistant Spore Pasteurization

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for performing highly heat resistant spore pasteurization for the determination of highly heat resistant spore counts in raw milk as well as 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

HHR SP- Highly Heat Resistant Spore Pasteurization

HHR MSC- Highly Heat Resistant Mesophilic Spore Count

HHR TSC- Highly Heat Resistant Thermophilic Spore Count

TC- temperature control

1.4 Safety

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



SECTION 2 MATERIALS

- Water bath capable of reaching and maintaining 100°C
- Ice
- Thermometer
- Timer
- Sterile 250 mL glass bottles with screw on cap if performing < 10 samples or if >35mL of sample is needed for plating
- Sterile 50mL glass vials with screw on cap if there are > 10 samples and 35mL of sample or less is needed for plating
- Temperature control glass bottle with hole in cap



SECTION 3 PROCEDURES

3.1. Sample preparation

3.2.1. To ensure uniform samples, shake 25 times in a 1-foot arc within 7 seconds prior to transferring sample to sterile bottles/vials in accordance with *Standard Methods for the Examination of Dairy Products* (Laird et al., 2004).

3.2.2. Aseptically transfer 100 mL (40 mL if using a sterile vial) of raw milk or pasteurized fluid milk, or hydrated powder product to a 250 mL or sterile glass bottle with screw cap. Use a 50mL sterile glass vial with a screw cap if there are fewer than 10 samples and if 35mL of sample is sufficient for plating

3.2.3. Prepare a TC with the same volume of raw, pasteurized or hydrated powder product to be processed, in a 250 mL glass bottle with hole in the screw cap for thermometer (or in a 50mL glass vial if vials are used)

3.2.4. Store samples and TC at 4°C until the water bath is at temperature

3.2. Highly Heat Resistant Spore Pasteurization

3.1.1. Set a water bath at 100°C and ensure the water level will exceed the level of the product in the bottle(s) or vial(s) by 4 mm.

3.1.2. Place samples and temperature control in water bath.

3.1.3. Start timing the 30-minute hold time when the temperature of the TC has reached 100°C. Maintain the temperature at 100°C ± 1°C for the duration of the hold time.

3.1.4. At the end of the hold time, immediately remove samples and TC from the water bath and place on ice.

3.1.5. When the temperature of the samples and TC cool to 10°C or lower proceed with sample analysis.

3.1.5.1. HHR SP treated samples plated and incubated at 32°C for 24-48 hours constitutes an HHR MSC.

3.1.5.2. HHR SP treated samples plated and incubated at 55°C for 24-48 hours constitutes an HHR TSC.



SECTION 4 TROUBLESHOOTING

- High protein powders (e.g., whey protein powder) may need to be diluted to 1:100 prior to performing the HHR heat treatment to avoid sample coagulation.

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. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.

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.

SECTION 6 METHOD VERSION & CHANGES

| VERSION | DATE | EDITOR | COMMENTS |
|-----------|------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------|
| Version 1 | 11/21/2011 | Nicole | Original SOP |
| Version 2 | 4/21/2020 | Rachel E. | Updated to new formatting and fixed grammatical errors. Added details for processing samples using 50mL sterile vials with a screw top |
| Version 3 | | | |