



Specially Thermoresistant Spore Enumeration

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for performing a specially thermoresistant spore enumeration for the determination of thermophilic spore counts in raw, pasteurized and powdered dairy products.

1.2 Scope

This SOP applies to the Milk Quality Improvement Program (MQIP) Lab

1.3 Definitions

STSE – Specially Thermoresistant Spore Enumeration

TC – Temperature control

SECTION 2 MATERIALS

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- **Modified pressure vessel capable of reaching and maintaining 106°C**
- **Ice**
- **Thermometer with probes**
- **Sterile 50 mL glass tubes with screw on cap**
- **Temperature control glass tubes with hole in cap**
- **Heating device, recommended induction hot plate**



SECTION 3 PROCEDURES

3.1. Sample preparation

- 3.2.1. In order to ensure uniform samples, shake 25 times in a 1 foot arc within 7 seconds prior to transferring sample to sterile tubes.
- 3.2.2. Aseptically transfer 20 mL of raw, pasteurized or rehydrated powder product to a 50 mL sterile glass tubes with screw cap. **DO NOT TIGHTEN CAPS FULLY DURING HEAT TREATMENT.**
- 3.2.3. Prepare a TC with the same volume of raw, pasteurized or hydrated powder product, or water if product is unavailable, to be processed, in a 50 mL glass tube.

3.2. STSE heat treatment

- 3.2.1. Place the pressure vessel, about half full with water, onto the heating device and bring water to a rolling boil.
- 3.2.2. Place samples (screw caps not fully tightened) and temperature control in a test tube rack and place carefully into the pressure vessel.
- 3.2.3. Place one temperature probe into the temperature control tube and allow the other temperature probe to settle into the chamber.
- 3.2.4. Close and lock the lid of the vessel and tighten the pressure valve to close
- 3.2.5. When the pressure reading goes above 3.5 PSI, turn down the temperature of the hot plate and open the pressure valve, being careful to avoid any escaping steam. Once the pressure has lowered to 3 PSI, partially close the valve and adjust until it settles at ~3.2 PSI.
- 3.2.6. When the temperature of the TC reaches 105.5°C, start the timer and maintain the temperature of the TC at 106°C ± 0.5°C for 30 minutes using the pressure valve and the temperature of the heat source.
- 3.2.7. Carefully remove the pressure vessel from the heat source and once the temperature of the vessel falls below 100°C open the lid and carefully remove the samples from the vessel and place on ice until at or below 6°C.

3.3. Plating STSE samples

- 3.3.1. Once cooled, shake or vortex the heat treated sample and spread plate up to 250 uL of appropriate dilutions onto prepared agar plates (e.g., plate count milk agar (PCMA)) in duplicate and incubate at 55°C for 48 hours followed by enumeration.

SECTION 4 TROUBLESHOOTING

SECTION 5 REFERENCES

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