



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



Title: **Specially Thermoresistant Spore Enumeration**

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## ***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 Food Safety Lab and the Milk Quality Improvement Program. The protocols may also be used by laboratory members from other locations.

### **1.3 Definitions**

STSE- Specially Thermoresistant Spore Enumeration

TC- Temperature Control

### **1.4 Safety**

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



## SECTION 2 MATERIALS

- 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**

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

3.1.2. 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.**

3.1.3. 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 of 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**

- A video SOP can be found at: <https://vimeo.com/296731234> password: sporemethods2018

**SECTION 5 REFERENCES**

*ISO-IDF. 2009. Dried milk: Enumeration of the specially thermoresistant spores of thermophilic bacteria. International Organization for Standardization (ISO), Geneva, Switzerland, and International Dairy Federation (IDF), Brussels, Belgium.*

**SECTION 6 METHOD VERSION & CHANGES**

<b>VERSION</b>	<b>DATE</b>	<b>EDITOR</b>	<b>COMMENTS</b>
Version 1	10/24/2018	Nicole	Original SOP
Version 2	4/23/20	Rachel E	Updated formatting of SOP