VSL Microbiological and Chemical Analysis

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for microbiologic and chemical analysis of Voluntary Shelf-Life (VSL) samples.

1.2 Scope

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

1.3 Definitions

PIC: Preliminary Incubation Count is a test that stresses pasteurized milk at 37°C for 6 hours prior to enumeration on coliform petri film to determine the presence of coliform.

PBC: Psychrotrophic Bacteria Count is a test that is designed to enumerate cold tolerant bacteria present in raw milk. This test is performed by plating raw milk on Standard Plate Count (SPC) media followed by a 10 day incubation at 7°C.

CVTA: Crystal Violet Tetrazolium Agar is a selective media designed to enumerate Gram-negative organisms in raw or pasteurized milk.

Initial Day: the initial day of testing is not tied to the pasteurization date of the product, it is simply the first day of testing.

1.4 Safety

SECTION 2 MATERIALS

- **Standard Plate Count (SPC) Agar.** Pre-poured SPC plates must be prepared and dried prior to use with the spiral plater.
- **Sterile 500mL Orange Top Bottles**. Bottles should be clean, sterile and free of condensation.
- **2oz Vials**
- **Coliform Petrifilms**
- **Ethanol**
- **Kimwipes**
- **Digital Thermometers**
- **Sterile 5mL Screw Capped Tubes**
- **Waterbath.** Set at 63°C
- **Incubators.** Set at 37°C, 32°C and 7°C
- **Spiral Plater**
- **Q-Count**
- **Quebec Colony Counter**
- **Hand Tallies**
- **Phosphate Buffer Dilution Blanks.** 900 µL and 99 mL volumes.
- **Variable Volume Pipettes.** 100-1000 µL volume and corresponding tips, 2mL fixed pipette with corresponding tips.
- **Vortex**

SECTION 3 PROCEDURES

3.1. Preparing For VSL

3.1.1. 1-2 Days Prior to Arrival of VSL Samples

3.1.1.1 Calculate the number of bottles, vials and plates you will need by checking the posted schedule. Tier 1 plants (and those with code dates >17 days) (bolded on VSL schedule) will need 5 bottles per core sample, all other plants will need 4 bottles per core sample. Each sample will need 4 vials, 2 pre-poured SPC plates, and two coliform petrifilms.

3.1.1.2 Label sample bottles with a piece of tape with the sample number written on it. Place the piece of tape at either the 300mL mark (Tier 1 plants (and those with code dates >17 days)) or the 400mL mark (all other plants).

3.1.1.3 Each sample needs **four vials** labeled with the sample number on the lid, one vial should have in addition to the sample number the letters PIC (Preliminary Incubation Count).

3.1.1.4 Label (in duplicate) pre-poured SPC plates and coliform petrifilms with the sample number, the day of plating (DI for day initial) and the dilution plated.

3.2. VSL Initial Day

3.2.1. Collecting Coolers and Logging in Plant Information

3.2.1.1 Wednesday morning collect samples from the cooler (Location TBD).

3.2.1.2 With the **lights turned off and blinds closed** (to minimize opportunity for sample to become light oxidized) in the pour off area, locate the temperature control (TC) associated with plant 1. In accordance with Standard Methods for the

Examination of Dairy Products (SMEDP 17th Edition) and using **aseptic technique** fully invert 25 times and wipe down the outside of the TC container and pour 400mLs into a labeled orange top TC bottle with temperature probe cap. Record temperature and time of reading on log sheet and place the control in the first 6°C incubator, noting the location of the TC bottle.

3.2.1.3 Before pouring off any of the samples check the sample label against the sample record sheet for sample ID, Sample type (confirm % fat on low fat containers), Container size and Type, Label, Code date and Package date and record the plant #.

3.2.2. Pouring off Core Samples

3.2.2.1 To ensure the temperature of the samples remains cold, set up the bottles and vials for each sample before removing it from the cooler. Pour off one sample at a time.

3.2.2.2 In accordance with Standard Methods for the Examination of Dairy Products (SMEDP 17th Edition) and using **aseptic technique** fully invert 25 times and wipe down the outside of the sample container and pour off 1 vial (to the vial line) for DI plating, leaving enough head space for mixing. Then pour enough sample to reach the pre-labeled volume into each of the labeled orange top bottles for that sample. Reserve enough sample to fill the remaining three vials for initial day sampling.

3.2.2.3 Check the empty sample container for any off odors, or physical defects (e.g. coagulation, particles, etc).

3.2.2.4 After pouring the sample into bottles and vials, move the orange top bottles to the 6°C refrigerator. Place two of the vials in racks in the bottom of the 6°C refrigerator, while the PIC vial and the remaining vial (micro vial for DI plating) should be placed in separate racks in the 4°C refrigerator.

3.2.2.5 Continue checking the VSL samples in, pouring off and refrigerating samples until all samples are poured off.

3.2.3. Pouring off and Processing Extras and Raw Samples

3.2.3.1 Label two 500mL bottles and three vials for each half-pint Extras sample. For each non half-pint Extras sample, label one 500mL bottle and three vials. Label three vials and a small sterile screw capped tube for each raw sample.

3.2.3.2 Check recorded sample information for each Extras and raw sample. Set aside two of the six half pint Extras containers for sensory.

3.2.3.3 The remaining four half pint Extras containers should be shaken and aseptically co-mingled in accordance with SMEDP. After co-mingling the sample, re-shake the sample and then aseptically pour off the sample into the vials, then split the remaining sample between the two 500 mL bottles. Non half-pint Extras should be poured off in the same manner as core samples.

3.2.3.4 Refrigerate the Extras sample bottles at 6°C for future sampling. Vials can be placed with the core sample vials for initial day testing.

3.2.3.5 Raw samples should also be shaken (25 times within 7 seconds in a 1-foot arc) and poured off aseptically according to SMEDP into the vials. Measure 5mL of each raw sample into screw cap tubes for the laboratory pasteurized (LP) test.

One additional tube should be prepared for use as a temperature control. These tubes should be kept on ice until performing the LP test.

3.2.3.6 Laboratory Pasteurization:

3.2.3.6.1 Turn the waterbath set at 63°C on several hours before beginning pasteurization.

3.2.3.6.2 Place all LP samples as well as the temperature control tube in a rack and place all in the waterbath. The level of the water in the bath should be at least 4 cm above the level of sample in the tubes.

3.2.3.6.3 Monitor the TC temperature, once the temperature has reached 63°C, set a timer for 30 minutes. During the pasteurization closely monitor the temperature of the samples.

3.2.3.6.4 At the end of the pasteurization remove the tubes from the bath and place on ice until TC reads 6°C. Samples may then be plated, or held at refrigeration temperature until plated.

3.2.3.7 In duplicate, label pre-poured SPC plates (undiluted and 1:100 dilutions) and coliform petrifilms (undiluted, 1:10and 1:100 dilutions) for all Extras and raw samples with the sample number, the day of plating (DI for day initial) and the dilution plated.

3.2.4 Plating Day Initial Samples

3.2.4.1 Remove cover from spiral plater. Rinse all three wells with 70% ethanol and fill the two water wells with sterile distilled water to between the black lines, and the disinfectant well with bleach to between the black lines.

3.2.4.2 Turn the spiral plater and the pump on. With the *Power Clean* setting selected press *Clean*.

3.2.4.3 After the completion of the power clean cycle, press the down arrow button to lower the stylus. Using a sample that has either been vortexed or shaken (SMEDP), carefully immerse the tip of the stylus into the sample in an area that is free from foam. Press either *Min* Fill (50uL) or *Max* Fill (250uL) being careful no to introduce air into the tubing by keeping the tip of the stylus immersed in the sample at all times. If the stainless tubing of the stylus touches the sample at any time, be sure to wipe the entire stylus down with 70% EtOH.

3.2.4.4 Once the sample has been taken up, position the pre-poured plate on the turntable, ensuring that the plate is firmly and centrally located. Be sure to line up the beginning mark on the turntable with the mark on the plate (if there is no mark on the side of the plate, use a marker to mark the beginning of the spiral).

3.2.4.5 Press *Plate*. While the sample is being distributed on the plate pay attention to the delivery. If any abnormalities or problems arise be sure to address them before continuing. After the sample has been completely plated the stylus will raise. Remove the plate from the turntable and replace the lid.

3.2.4.6 Load the duplicate plate and press *Plate* again for the second plate.

3.2.4.7 After the sample has been plated, clean the spiral plater by pressing the *Clean* button (the Power Clean setting may be used at any time, but generally it does not need to be used during plating except at the beginning and end of use.).

3.2.4.8 Once all samples have been plated using this method, Power Clean the spiral plater, turn the power and the pump off and replace the cover on the wells and on the machine.

3.2.4.9 In addition to the sample plating, buffer, Air Density (AD) and agar controls must also be plated in conjunction with sample plating.

3.2.4.10 Incubate all Aerobic Plate Count (APC), LP SPC plates, all control plates and coliform petrifilms at 32°C for 48 hours and 24 hours respectively. Incubate PBC plates at 7°C for 10 days.

3.2.5 Day Initial Chemistry

3.2.5.1 All sample vials for butterfat analysis should be labeled and taken to Dr. Barbano's staff for analysis.

3.2.5.2 All sample vials for cryoscope analysis should be labeled and taken to DairyOne for analysis.

3.2.6 Paperwork

3.2.6.1 After the original VSL log sheet has been checked and the information is correct and up to date, make a schedule of remaining test days. This is done by calculating days 7, 10, 14, and 17 (if applicable) from the date the milk was processed (day 0).

3.2.6.2 Make copies of the schedule and the log sheet for all team members involved in VSL. Attach original to the back of the core worksheets.

3.3 VSL Day 1

3.3.1 Preliminary Incubation Count for Coliform

3.3.1.1 Thursday morning place all PIC vials of core samples into the 37°C incubator in room 200. Set a timer for 6 hours.

3.3.1.2 After the incubation is complete use the electronic pipettor to plate samples (undiluted, 1:10 and 1:100) in duplicate on coliform petrifilms.

3.3.2 Counting

3.3.2.1 Count coliform petrifilms after 24 hours (+/- 1 hour) of incubation at 32°C.

3.3.2.2 Only count colonies that are red and are associated with an air bubble.

3.3.2.3 If films are Too Numerous To Count (TNTC) (i.e., dark red/purple color develops on the film, but no individual colonies with gas production are obvious), a Brilliant Green Bile Broth (BG) tube may be preformed to confirm the presence of coliform. BG tubes should be incubated at 32°C for 48 hours and checked for acid and gas production.

3.3.2.4 If a TNTC plate is confirmed (either by gas production, but no individual colonies, or by BG confirmation), the final count is >150 estimate (E). This count is raised to the power of the highest dilution plated that was TNTC.

3.3.2.5 Coliform petrifilms with no colonies present should be recorded and reported as <1 cfu/mL (detection limit), and those that are TNTC should be recorded and reported as >150 cfu/mL.

3.3.2.6 An estimate may be done of the film that is very crowded by counting the confirmed colonies in one square and multiplying that number by 20 to obtain the estimated number of colonies on the entire film.

3.4 VSL Day 2

3.4.1 Counting

3.4.1.1 Remove all SPC (APC, LP, Controls) plates and PIC coliform petrifilms from the 32°C incubator.

3.4.1.2 Count PIC's in the same manner as described above (VSL Day 1).

3.4.1.3 Count all other plates using the Q-Count equipment.

3.4.1.4 Count plates in sequential order by group (core, schools, raws). When naming the sample in the program include the sample ID, the day of plating and the test type (ie, 11 SPC DI or 52 CVTA D14).

3.4.1.5 For more detailed procedures for counting plates using the Q-Count consult the manual located on the 4th floor.

3.4.1.6 Record the dilutions used for each test and the plate counts on the VSL worksheets. Plates with a SPC count of less than 400 should be recorded as estimate (E) on the worksheet final count. SPC plates that are TNTC (spiral plate won't give an estimate), should be recorded as >400,000 E to the power of the dilution used. SPC plates with no colonies present should be recorded as less than the detection limit (<10 cfu/mL).

3.4.1.7 Round final counts to two significant figures. If the third place number is a 5, there are two options. If the second place number is even, the number should remain as it is (i.e., 12.5 would round to 12). If the second place number is odd, round the number up (i.e., 135 would round to 140).

3.4.1.8 When reporting counts, all samples with SPC counts of <400 cfu/mL or < detection limit should be reported as <400 cfu/mL. All samples with TNTC SPC counts should be reported as >400,000 E to the power of the dilution used.

3.4.2 Entering Data into Access

3.4.2.1 Once initial day plates and petrifilms are counted, and the chemistry results are done, enter this data into the VSL Access database.

3.4.3 Checking Initial Day Reports

3.4.3.1 Completed reports can be generated for core, school and raw samples after the data is in the database.

3.4.3.2 Micro, Sensory and Chemistry data on the reports must be checked for accuracy against the original worksheets. If a mistake is found on the worksheet or on the reports correct it.

3.5 Additional VSL Days

3.5.1 Pouring off samples

3.5.1.1 Samples are tested according to the table below:

	Day Initial	Day 1	Day 7	Day 10	Day 14	Day 17*	Day 21*
Raw SPC	X						
Raw PBC	X						
Raw LP	X						
Raw Coliform	X						
Core SPC	X		X	X	X	X	X
Core Coliform	X		X	X	X	X	X
Core Sensory	X			X	X	X	X
Core CVTA					X		
Core PIC		X					
Extra SPC	X		X	X	X	X	X
Extra Coliform	X		X	X	X	X	X
Extra Sensory**		X			X		
Extra CVTA					X		

* Only Tier 1 plants and Tier 2 plants with >17d code dates will be tested at day 17 and day 21

** Sensory only performed on 2% and chocolate half-pint extras

3.5.1.2 Samples must be ready for sensory by 9am on VSL testing days 10, 14, 17 and 21 (only NYS tier 1 plants and those plants with >17d code dates will be tested for sensory at day 17 and day 21).

3.5.1.2.1 Day 7 micro sample should be poured off from the day 10 Sensory bottle.

3.5.1.3 Verify that all samples being tested have <10 cfu/mL coliform. If a sample has ≥10 cfu/mL coliform, inform the person responsible for sensory and DO NOT send the sample for sensory testing.

3.5.1.4 With the lights turned off gently invert the bottles 25 times before aseptically filling a labeled 2oz vial with sample.

3.5.1.5 When all samples are poured off into vials, place the remaining sample in the bottles on the door of the 6°C refrigerator for sensory.

3.5.1.6 Vials should be refrigerated until used for micro testing.

3.5.1.7 Perform SPC and coliform testing as described above at appropriate dilutions (on days 7 and beyond, plate two different dilutions that are at least one dilution apart to ensure plates will be countable; i.e., -1 and -3 dilution) in duplicate to ensure countable plates (i.e., a 1:10 and 1:1,000 dilution done to cover a larger number of dilutions).

3.5.1.8 CVTA counts should be performed on day 14 for all samples. Spiral plate undiluted milk onto pre-poured CVTA media. Incubate for 48 hours at 21°C before counting red colonies.

3.5.1.9 Counting and data entry as described above.

SECTION 4 TROUBLESHOOTING

- In the case that a sample arrives frozen leave the sample in the 6°C incubator until the sample is completely free of ice before pouring off.

SECTION 5 REFERENCES

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.