BACTOSCAN™ 5
(Raw Commingled Cow Milk Only)
IMS #7f

[Unless otherwise stated all tolerances are ±5%]

GENEREAL REQUIREMENTS

1. Cultural Procedures (CP) items 1-33, as appropriate _____

2. Sample Requirements, see CP items 34 and 35 [For inhibitor testing requirements, refer to Section 7 of the PMO] _____

a. Raw milk tested only _____

PRE-REQUISITE

3. Comparative Test _____

a. Test 25 samples in duplicate using the SPC (2400a), PAC or RAC (2400a-4) and BactoScan 5 (BSC 5) methods _____

b. Comparisons done by each certified analyst performing test _____

1. Results must be shown to be acceptable before official tests may be performed by the analyst _____

c. Copy of comparison and results in QC record (or easily accessible file in laboratory); kept for as long as analyst is certified _____

d. Analysts certified for SPC, PAC or RAC methods _____

e. Alternatively, a BactoScan Industry Operator (BIO) can analyze samples for regulatory compliance _____

1. Industry operator must complete the BIO operating protocols, training and oversight. Maintain records _____

2. Laboratory must maintain at least one certified BactoScan analyst (item 3.a-d.) for training and ongoing oversight of the BIO _____

3. Refer to BIO approved training procedures _____

4. Maintain records for all BIO oversight _____

APPARATUS

4. BactoScan 5 (BSC 5) Model _____

a. BSC 5 65 (speed 65 samples per hour) _____ _____

b. BSC 5 100 (speed 100 samples per hour) _____ _____

c. BSC 5 130 (speed 130 samples per hour) _____ _____

d. BSC 5 150 (speed 150 samples per hour) _____ _____

e. BSC 5 200 (speed 200 samples per hour) _____ _____

REAGENTS

5. Purified Water, deionized (conductivity less than 2 µS/cm, see CP item 24.c.3) and filter sterilized with a 0.2 µm filter _____

6. BactoScan 5 Reagents Supplied by Manufacturer _____

a. Buffer Powder, package Lot #: _____ Exp. Date: _____ _____

b. Detergent, bottle Lot #: _____ Exp. Date: _____ _____

c. Staining Medium, bottle Lot #: _____ Exp. Date: _____ _____

d. Enzyme 150 Lot #: _____ Exp. Date: _____ _____

e. Bacterial Control Sample (BCS Control) Lot #: _____ Exp. Date: _____ _____

f. Particle Control Sample (PCS Control) Lot #: _____ Exp. Date: _____ _____

g. Rinse/Sheath Concentrate Lot #: _____ Exp. Date: _____ _____

h. Rehydration Kit Lot #: _____ Exp. Date: _____ _____

1. Rehydration Powder Lot #: _____ Exp. Date: _____ _____

2. Rehydration Tablets Lot #: _____ Exp. Date: _____ _____

7. BactoScan Reagent Filter _____

8. All Chemicals not Provided by Manufacturer, Analytical Grade _____

9. Solutions _____

a. Staining Solution (Options for a 10 liter or 5 liter batch size) _____

1. High volume testing, 10 liter batch (approx. 7000 samples) _____

a. Measure approx. 8 liters of purified water (item 5) into a 10 liter container and carefully add one Buffer Powder package (item 6.a) _____

b. Stir mixture on a stir plate until the powder is completely dissolved (approx.. 15 minutes); Optionally, to speed up the process, heat to 40°C while stirring _____

c. Slowly (to avoid foaming) add one bottle of thoroughly shaken Detergent (item 6.b) and stir well (approx. 3-5 minutes) _____

d. Add one bottle of Staining Medium (item 6.c) and stir well (approx. 3-5 minutes) _____

e. Slowly fill up to the 10 liter (± 2%) mark with purified water (item 5) and stir well (approx. 3-5 minutes) _____

f. Store in the dark for up to 8 weeks at room temperature (15-25°C); do not refrigerate _____

Lab Prep. Date: _____ Exp. Date: _____ _____

2. Low volume testing, 5 liter batch (approx. 3000 samples) _____

a. To prepare Buffer Solution, measure approximately 8 liters of purified water (item 5) into a 10 liter container and carefully add one Buffer Powder package (item 6.a) _____

b. Stir mixture on a stir plate until the powder is completely dissolved (approx. 15 minutes); Optionally, to speed up the process, heat to 40°C while stirring _____

c. Fill up to the 9 liter (± 2%) mark with purified water (item 5) and stir well (approx. 3-5 minutes) _____

d. Store Buffer Solution in the dark for up to 20 weeks at room temperature (15-25°C) _____

Lab Prep. Date: _____ Exp. Date: _____ _____

e. Measure 4.5 liters of Buffer Solution (item 9.a.2.d) into a 5 liter container. Slowly (to avoid foaming) add 250 mL (± 10mL) of thoroughly shaken Detergent (item 6.b) and stir well (approx. 3-5 minutes) _____

f. Add 50 mL (± 2 mL) of Staining Medium (item 6.c) and stir well (approx. 3-5 minutes) _____

g. Slowly fill up to the 5 liter (± 2%) mark with purified water (item 5) and stir well (approx. 3-5 minutes) _____

h. Store Staining Solution in the dark for up to 8 weeks at room temperature (15-25°C); do not refrigerate _____

Lab Prep. Date: _____ Exp. Date: _____ _____

b. Rehydration Solution for Bacterial Control Sample (item 6.e), as the Blank Solution (item 10.f), and media for the Particle Control Sample (item 6.f) _____

1. Measure approx. 4.5 liters of purified water (item 5) into a 5 liter container and add one container of Rehydration Powder (item 6.h.1) _____

2. Add 10 Rehydration Tablets (item 6.h.2) _____

3. Stir until completely dissolved using stir plate _____

4. Fill up to the 5 liter (± 2%) mark with purified water (item 5) _____

5. Store at 0.0 to 21°C for up to 4 weeks _____

Lab Prep. Date: _____ Exp. Date: _____ _____

c. Rinse/Sheath Solution _____

1. Invert Rinse/Sheath Concentrate (item 6.g) bottle 5 times. Pour 100 mL Rinse/Sheath Concentrate then add 50 liters purified water (item 5) to ensure complete mixing of the two liquids _____

2. Mix thoroughly _____

3. Store at room temperature (15-25°C) for a maximum of 14 days _____

Lab Prep. Date: _____ Exp. Date: _____ _____

d. End of Day Solution _____

1. Pour 10 liters (±10%) of purified water (item 5) and add 50 mL (± 10%) Ammonia (25% analytical grade) _____

2. Mix thoroughly _____

3. Store at room temperature (15-25°C) for a maximum of 7 days _____

Lab Prep. Date: _____ Exp. Date: _____ _____

e. Bacterial Control Sample (BCS) (item 6.e) _____

1. Measure 100 mL (± 2%) of Rehydration Solution (item 9.b) and transfer it to a suitable container with a lid _____

2. Take a Bacterial Control Sample vial (item 6.e) from the freezer _____

a. Remove the metal cap and loosen the lid _____

b. Use a small sterile, disposable 5 mL pipette to transfer 2-3 mL of the Rehydration Solution (item 9.b) from the container (item 9.e.1) into the BCS vial _____

c. Close the BCS vial and shake to completely dissolve _____

d. Refill the pipette with clean Rehydration Solution (item 9.b) from the container (item 9.e.1) _____

3. When the Control Sample is dissolved, pour the contents of the BCS vial into the container (item 9.e.1) _____

a. Use the contents of the pipette (item 9.e.2.d) to rinse the BCS vial _____

b. Pour the contents of the BCS vial into the container (item 9.e.1) _____

4. Put on the lid and shake well _____

5. Store in a refrigerator (0.0-4.5°C) except when filling sample vials _____

6. The re-constituted, preserved Bacterial Control Sample can be stored for up to 10 hours when kept in the refrigerator (0.0-4.5°C) _____

Lab Prep. Date: _____ Lab Prep. Time: _____ _____

f. Blank Solution _____

1. Rehydration Solution (item 9.b) is used as the Blank Solution _____

Lab Prep. Date: _____ Exp. Date: _____ _____

10. All Solution Containers Labeled with Solution Name, Date Prepared and Expiration Date (when relevant) _____

START-UP

11. Daily Instrument Start-up _____

a. Replace the used incubation reagent filter (item 7) on the intake assembly _____

1. Turn the handle that holds the filter in position _____

2. Remove and discard the old filter after 24 hours _____

3. Insert the new filter and release the handle _____

b. Check the Staining Solution container (item 9.a.1 or 9.a.2) and fill up if required (Previous day's solution can be used) _____

1. Check expiration date (item 9.a.1.f or 9.a.2.h) _____

2. Staining Solution must be completely replaced, leftover discarded, when expired (item 9.a.1.f or 9.a.2.h) _____

c. Check the Rinse/Sheath Solution container (item 9.c) and fill up if required (Previous day's solution can be used) _____

1. Check expiration date (item 9.c.3) _____

2. Rinse/Sheath Solution must be completely replaced, leftover discarded, every 14 days (item 9.c.3) _____

d. Transfer the Rinse/Sheath and Staining Solution probes from the End of Day Solution to the appropriate liquid containers, note correct probe for each liquid _____

e. Check the Enzyme 150 Bottle (item 6.d) and replace if necessary _____

1. Check expiration date (item 6.d) and replace if expired _____

f. Switch the system on _____

g. Prepare Bacterial Control Sample (BCS) (item 9.e) _____

1. Store in refrigerator (0.0-4.5°C) until used _____

2. Refer to item 9.e for rehydration procedure _____

h. Prepare rack with a Control Sample Batch Rack _____

1. 4 Blanks (item 9.f), 1 BCS (item 9.e), 4 Blanks (item 9.f) _____

i. Enter (or use) appropriate batch type, with correct sample types (i.e., Blank and BCS) _____

1. This will ensure the correct presentation and calculation of results _____

2. Check lot number to see that it corresponds with the lot being tested _____

j. Measure the Control Sample Batch Rack (item 11.h) at the start and end of each run. Additionally, run the Control Sample Batch Rack every hour throughout the working session _____

k. When the Control Sample Batch Rack has been measured: _____

1. Check that blank counts are within acceptable limits, all results ≤1 CFU. Evaluate vials 2-4 and 7-9 _____

2. Check that the results of the Bacterial Control Sample (item 9.e) conform to the specified limits (vial 5). The Laboratory Average Count must be within the Manufacturer Count Limits and the Laboratory Average Signal Mean must be within the Manufacturer Provided Average Signal Mean (±2) _____

Manufacturer Provided Average Count _____ _____

Manufacturer Provided Count Limits _____ _____

Laboratory Average Count _____ _____

Manufacturer Provided Average Signal Mean _____ _____

Laboratory Average Signal Mean _____ _____

a. If the BCS sample is outside the specified limits, and does not correct after re-measurement, seek technical assistance _____

3. The Control Sample Batch Rack can be reused up to 10 hours with acceptable results, when maintained at 0.0-4.5°C _____

l. If any of the above parameters are “Out of Range” and do not correct after re-measurement, seek technical assistance _____

m. Do not proceed with sample counting if any parameters are out of specification _____

n. Records to be maintained on all parameters each time instrument is used _____

PROCEDURE

12. Handling Samples _____

a. Samples must first be tested for the presence of inhibitors before run on the BactoScan 5 _____

b. Samples kept at 0.0-4.5°C until tested _____

13. Testing Samples _____

a. Before placing the samples in racks, invert them 10 times to mix, or place samples in rack and invert rack with samples 10 times to mix _____

b. Place rack on conveyor and start the automatic testing procedure immediately _____

c. Samples run on the BactoScan 5 may be immediately placed into a 37-42°C water bath to run for ESCC _____

d. Alternatively, refer to CP item 34.a.7.a.1 _____

14. Results _____

a. The readout is in IBC (Individual Bacteria Counts)/ µL _____

b. IBC is converted using the conversion table pre-programmed into the instrument and is reported in the result list as CFU/µL _____

c. Proper conversion factor has been entered for the regulatory range _____

15. Records _____

a. Maintain records of all results, controls and samples _____

16. Follow End of Day Shut-Down and Cleaning _____

a. Check the End of Day Solution container (item 9.d) and refill if required (Previous day's solution can be used) _____

b. Check expiration date of End of Day Solution (item 9.d.3) _____

c. End of Day Solution must be completely replaced when expired (item 9.d.3) _____

d. Place the BactoScan 5 probes for Rinse/Sheath Solution and Staining Solution (both) into the End of Day container _____

e. Proceed with the shut-down procedure _____

REPORTING

17. Reporting _____

a. Report the bacterial content of the milk as BSC 5 CFU/mL (CFU/µL x 1000 = CFU/mL) _____

1. Instrument reports in CFU/µL, laboratory analyst must convert to CFU/mL for official reporting _____

b. Report only first two left-hand digits _____

1. If the third digit is 5 round the second number using the following rules _____

a. When the second digit is odd round up (odd up, 235 to 240) _____

b. When the second digit is even round down (even down, 225 to 220) _____

c. If presence of inhibitor is detected, colony count cannot be reported, report as inhibitor found (IF) _____