BACTOSCAN™ FC/FC+
(Raw Commingled Cow Milk Only)
IMS #7a

[Unless otherwise stated all tolerances are ±5%]

GENERAL REQUIREMENTS

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

2. Sample Requirements, see CP items 34 & 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 FC/FC+ (BSC FC/FC+) 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.b.c.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 FC (BSC FC/FC+) Model _____

a. BSC FC 50 H (speed 50 samples per hour) ____ _____

b. BSC FC 100 H (speed 100 samples per hour) ____ _____

c. BSC FC 150 H (speed 150 samples per hour) ____ _____

d. BSC FC+ 65 H (speed 65 samples per hour) ____ _____

e. BSC FC+ 130 H (speed 130 samples per hour) ____ _____

f. BSC FC+ 200 H (speed 200 samples per hour) ____ _____

REAGENTS

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

6. BactoScan 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 50 or 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 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. Stock Solutions _____

a. Sheath Liquid Stock Solution _____

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

2. Stir mixture thoroughly on a stir plate until the powder is completely dissolved; Optionally, to speed up the process, heat to 40°C while stirring. Add one bottle of Detergent (item 6.b) and stir well _____

3. Slowly (to avoid foaming) fill to the 10 liter (± 2%) mark with purified water (item 5) _____

4. Store for up to 6 weeks at room temperature (15-25˚C); do not Refrigerate _____

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

b. Staining Reagent Stock Solution _____

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

2. Stir mixture on a stir plate until the powder is completely dissolved _____

3. Slowly (to avoid foaming) add one bottle of Staining Medium (item 6.c), one bottle of Detergent (item 6.b), and stir well. Fill up to the 10 liter (± 2%) mark with purified water (item 5) _____

4. Store in the dark for up to 6 weeks at room temperature (15-25˚C); do not refrigerate _____

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

c. Preservation Stock Solution for Bacterial Control Sample (required for Rehydration Solution preparation item 9.d.1) _____

1. Add 53g Boric Acid, 0.8g Potassium Sorbate and 10g Glycerol into a 2 liter container _____

2. Fill up to the 2000 mL mark with purified water (item 5) _____

3. Stir on stir plate until completely dissolved; Optionally, to speed up the process, heat to 40°C while stirring _____

4. Store at room temperature (15-25˚C) for up to 10 weeks _____

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

d. Rehydration Solution _____

1. Option 1: Rehydration Solution prepared using Preservation Stock Solution (item 9.c); used for the Bacterial Control Sample (item 6.e) _____

a. Add one Ringer Tablet (Code BR 52) into a 1 liter container _____

b. Add 300 mL purified water (item 5) and 200 mL Preservation Stock Solution (see item 9.c) _____

c. Stir until completely dissolved using stir plate _____

d. Store at room temperature (15-25˚C) for up to 7 days _____

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

2. Option 2: Rehydration Solution prepared using the Rehydration Kit (item 6.h); used for the Bacterial Control Sample (item 6.e), as the Blank Solution (item 10.f), and media for the Particle Control Sample (item 6.f) _____

a. 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) _____

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

c. Stir until completely dissolved using stir plate _____

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

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

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

10. Ready to Use Solutions _____

a. Ready to Use Sheath Solution _____

1. Pour 8 liters (± 10%) of purified water (item 5) and 2 liters (± 10%) of Sheath Liquid Stock Solution (item 9.a) into a 10 liter container _____

2. Replace lid and invert 10 times to mix thoroughly _____

3. Store at room temperature (15-25˚C) up to 7 days or 25-35°C up to 2 days _____

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

b. Ready to Use Blank Solution _____

1. Option 1: _____

    a. Mix 1 liter (± 10%) of purified water (item 5) and 50 mL (± 10%) of Sheath Liquid Stock Solution (item 9.a) in a sterilized 1 liter container and invert 10 times to mix well _____

    b. Use on day of preparation _____

    Lab Prep. Date: _____ _____

2. Option 2: The Rehydration Solution (item 9.d.2) is used as the Blank Solution _____

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

c. Ready to Use End of Day Solution _____

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

2. Invert 10 times to mix well _____

3. Store at room temperature (15-25˚C) for maximum of 7 days (discard left over solution and make up fresh solution) _____

d. Ready to Use Rinse Solution _____

1. Pour 100 mL Rinse Concentrate (item 6.g) into a 50 liter container, 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 maximum of 7 days _____

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

e. Ready to Use Incubation Reagent _____

1. For 150/200 samples/hr, mix 1600 mL (± 2%) of Staining Reagent (item 9.b) with 1 bottle of Enzyme 150 (item 6.d) _____

2. For 100/130 samples/hr, mix 1100 mL (± 2%) of Staining Reagent (item 9.b) with 2 bottles of Enzyme 50 (item 6.d) _____

3. For 50/65 samples/hr, mix 550 mL (± 2 %) of Staining Reagent (item 9.b) with 1 bottle of Enzyme 50 (item 6.d) _____

4. Invert container 10 times to mix thoroughly before use _____

5. Must be used on day of preparation, discard any leftovers _____

Lab Prep. Date: _____ _____

f. Ready to Use Bacterial Control Sample (BCS) (item 6.e) _____

1. Measure 100 mL (± 2%) of Rehydration Solution (item 9.d.1 or 9.d.2) 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.d.1 or 9.d.2) from the container (item 10.f.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.d.1 or 9.d.2) from the container (item 10.f.1) _____

3. When the Control Sample is dissolved, pour the contents of the BCS vial into the container (item 10.f.1) _____

a. Use the contents of the pipette (item 10.f.2.d) to rinse the BCS vial _____

b. Pour the contents of the BCS vial into the container (item 10.f.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: _____ _____

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

START-UP

12. Daily Instrument Start-up _____

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

1. Lift the spring-loaded disc that holds the filter in position _____

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

3. Insert the new filter and release the disc _____

b. Prepare Incubation Reagent (item 10.e) fresh daily _____

c. Prepare Sheath Reagent (item 10.a) _____

1. Check expiration date _____

2. Sheath Reagent must be completely replaced when expired (item 10.a.3) _____

d. Check the large container for Rinse Solution (item 10.d) and fill up if required (Previous day's solution can be used) _____

1. Check expiration date (item 10.d.3) _____

2. Rinse Solution must be completely replaced, leftover discarded, every 7 days, see item 10.d.3 _____

e. Transfer the Rinse and Incubation Reagent probes from End of Day Solution to the appropriate liquid containers, note correct probe for each liquid _____

f. Switch the system on _____

g Prepare Bacterial Control Sample (BCS) (item 10.f) _____

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

2. See item 10.f for rehydration procedure _____

h. Prepare rack with a Control Sample Batch Rack _____

1. 4 Blanks (item 10.b.1 or 10.b.2), 1 BCS (item 10.f), 4 Blanks (item 10.b.1 or 10.b.2) _____

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

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 12.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 10.f) 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

13. Handling Samples _____

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

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

14. 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 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 _____

15. Results _____

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

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

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

16. Records _____

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

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

a. Check the End of Day Solution container (item 10.c) and refill if required (Previous day's solution can be used) _____

b Check expiration date of End of Day Solution (item 10.c.3) _____

c. End of Day Solution must be completely replaced when expired (item 10.c.3) _____

d. Place the BSC FC/FC+ probes for Incubation Reagent and Rinse Solution (both) into the End of Day container, leave the Sheath Liquid probe in the Sheath Liquid container _____

e. Proceed with the shut-down procedure _____

REPORTING

18. Reporting _____

a. Report the bacterial content of the milk as BSC FC/FC+ 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) _____