

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

[Unless otherwise stated all tolerances are ±5%]

GENERAL REQUIREMENTS

1. Cultural Procedures (CP) items 1-32, as appropriate _____

2. Sample Requirements, see CP items 33 & 34 _____

a. Raw milk tested only _____

PRE-REQUISITE

3. Comparative Test _____

a. Test 25 samples in duplicate using the SPC (2400a) or PAC (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) _____

d. Analysts certified for SPC or PAC 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 _____

4. Monitoring of Regulatory Cut-Off Level _____

a. Select 10 samples counting between 150,000 and 450,000 IBC/mL (50,000 and 150,000 CFU/mL) each month _____

- b. Test each of these samples in duplicate (same dilution) using SPC or PAC and BSC FC/FC+ _____
- c. Report paired results (CFU/mL and IBC/uL) as specified by the FDA _____

APPARATUS

5. 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

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

7. BactoScan Reagents Supplied by Manufacturer _____

- a. Buffer Powder, package Lot #: _____ Exp. Date: _____
- b. Detergent, 500 mL bottle Lot #: _____ Exp. Date: _____
- c. Staining Medium, bottle Lot #: _____ Exp. Date: _____
- d. Enzyme 50 or Enzyme 150 Lot #: _____ Exp. Date: _____
- e. Bacterial Control Sample Lot #: _____ Exp. Date: _____
 (BCS Control)
- f. Particle Control Sample Lot #: _____ Exp. Date: _____
 (PCS Control)
- g. Rinse Concentrate Lot #: _____ Exp. Date: _____

8. BactoScan Reagent Filter _____

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

10. Stock Solutions

a. Sheath Liquid Stock Solution

1. Measure approx. 8 liters of purified water (item 6) into a 10 liter container and add one Buffer Powder package (item 7.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. Then add 500 mL (one bottle) of detergent (item 7.b)
3. Slowly (to avoid foaming) fill to the 10 liter ($\pm 2\%$) mark with purified water (item 6)
4. Store for up to 6 weeks at room temperature, do not refrigerate

Lab Prep. Date: _____ Exp. Date: _____

b. Staining Reagent Stock Solution

1. Measure approx. 8 liters of purified water (item 6) into a 10 liter container and carefully add one Buffer Powder package (item 7.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, 500 mL of Detergent (one bottle) (item 7.b) and fill up to the 10 liter ($\pm 2\%$) mark with purified water (item 6)
4. Store in the dark for up to 6 weeks at room temperature; do not refrigerate

Lab Prep. Date: _____ Exp. Date: _____

c. Preservation Stock Solution for Bacterial Control Sample

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 6)
3. Stir on stir plate until completely dissolved, to speed up process, heat to 40°C while stirring
4. Store at room temperature ($< 25^\circ\text{C}$) for up to 10 weeks

Lab Prep. Date: _____ Exp. Date: _____

- d. Re-hydration Solution for Bacterial Control Sample (item 7.e) _____
1. Add one Ringer Tablet (Code BR 52) into a 1 liter container _____
 2. Add 300 mL purified water (item 6) and 200 mL Preservation Stock Solution (see item 10.c) _____
 3. Stir until completely dissolved using stir plate _____
 4. Store at room temperature (< 25°C) for up to 7 days _____
- Lab Prep. Date: _____ Exp. Date: _____

11. Ready to Use Solutions _____

- a. Ready to Use Sheath Solution _____
1. Pour 8 liters ($\pm 10\%$) of purified water (item 6) and 2 liters ($\pm 10\%$) of Sheath Liquid Stock Solution (item 10.a) into a 10 liter container _____
 2. Replace lid and invert 10 times mix thoroughly _____
 3. Store at room temperature (< 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. Mix 1 liter ($\pm 10\%$) of purified water (item 6) and 50 mL ($\pm 10\%$) of Sheath Liquid Stock Solution (item 10.a) in a sterilized 1 liter container and invert 10 times to mix well _____
 2. Use on day of preparation _____
- Lab Prep. Date: _____

- c. Ready to Use End of Day Solution _____
1. Pour 10 liters ($\pm 10\%$) of purified water (item 6) and add 50 mL ($\pm 10\%$) Ammonia (25% analytical grade) _____
 2. Invert 10 times to mix well _____
 3. Can be stored at room temperature (< 25°C) for maximum of 7 days (discard left over solution and make up fresh solution) _____
- Lab Prep. Date: _____ Exp. Date: _____

d. Ready to Use Rinse Solution _____

1. Pour 100 mL Rinse Concentrate (item 7.g) into a 50 liter container first, then add 50 liters purified water (item 6) to ensure complete mixing of the two liquids _____
2. Mix thoroughly _____
3. Can be stored at room temperature (< 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 ($\pm 2\%$) of Staining Reagent (item 10.b) with 1 bottle of Enzyme 150 (item 7.d) _____
2. For 100/130 samples/hr, mix 1100 mL ($\pm 2\%$) of Staining Reagent (item 10.b) with 2 bottles of Enzyme 50 (item 7.d) _____
3. For 50/65 samples/hr, mix 550 mL ($\pm 2\%$) of Staining Reagent (item 10.b) with 1 bottle of Enzyme 50 (item 7.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 7.e) _____

1. Measure 100 mL ($\pm 2\%$) of Re-hydration Solution (item 10.d) and transfer it to a suitable container with a lid _____
2. Take a Bacterial Control Sample 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 Re-hydration Solution (item 10.d) into the Vial _____
 - c. Close the vial and shake to completely dissolve _____
 - d. Refill the pipette with clean Re-hydration Solution (item 10.d) _____
3. When the Control Sample is dissolved, pour the contents of the vial into the container (item 11.f.1) _____
 - a. Use the contents of the pipette (item 11.f.2.d) to rinse the vial _____

- b. Pour the contents of the vial into the container with the dissolved Control Sample _____
- 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: _____

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

START-UP

13. Daily Instrument Start-up _____

- a. Replace the used incubation reagent filter (item 8) 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. Remove the End of Day solution container _____
- c. Prepare Incubation Reagent (item 11.e) fresh daily _____
- d. Prepare Sheath Reagent (item 11.a) _____
 - 1. Check expiration date _____
 - 2. Sheath Reagent must be completely replaced when expired (item 11.a.3) _____
- e. Check the large container for Rinse Solution (item 11.d) and fill up if required (Previous day's solution can be used) _____
 - 1. Check expiration date (item 11.d.3) _____
 - 2. Rinse Solution must be completely replaced, leftover discarded, every 7 days, see item 11.d.3 _____
- f. Transfer the Rinse and Incubation Reagent probes from End of Day Solution to the appropriate liquid containers, note correct probe for each liquid _____

g. Switch the system on _____

As the instrument warms up

h. Prepare Bacterial Control Sample (BCS) (item 11.f) _____

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

2. See item 11.f for re-hydration procedure _____

i. Prepare rack with a Control Sample Batch Rack _____

1. 4 Blanks (item 11.b), 1 BCS, 4 Blanks (item 11.b) _____

j. 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 _____

k. Measure the Control Sample Batch Rack (item 13.i) at the start and end of each run. Additionally run the Control Sample Batch Rack every hour throughout the working session _____

l. 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 11.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

m. If any of the above parameters are "Out of Range" and do not correct after re-measurement, seek technical assistance

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

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

PROCEDURE

14. 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

15. 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 33.a.7.a.1

16. Results

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

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

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

17. Records

a. Maintain records of all results, controls and samples

18. Follow End of Day Shut-Down and Cleaning

- a. Place the BSC FC/FC+ probes for Incubation Reagent and Rinse (both) into the End of Day container, leave the Sheath Liquid probe in the Sheath Liquid container
- b. Proceed with the shut-down procedure

REPORTING

19. Reporting

[When samples are demonstrated to contain inhibitors, no bacteria counts are reported; report as positive for inhibitors or growth inhibitors (GI)]

- a. Report the bacterial content of the milk as BSC FC/FC+ CFU/mL (CFU/uL x 1000 = CFU/mL)
 - 1. Instrument reports in CFU/uL, 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)