BENTLEY BACTOCOUNT IBC
(Raw Commingled Cow Milk Only)
IMS #7c

[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 testing only _____

3. Maintenance Requirements _____

a. Confirm that the Annual Preventive Maintenance check has been completed within the last 12 months _____

Date of Last Check: _____ _____

PRE-REQUISITE

4. Comparative Test _____

a. Test 25 samples in duplicate using the SPC (2400a), PAC or RAC (2400a-4), or PPAC (2400a-6) and BactoCount IBC (BCC) 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, RAC, or PPAC methods _____

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

1. Industry operator must complete the BCIO operating protocols, training and oversight. Records maintained _____

2. Laboratory must maintain at least one certified BactoCount analyst (items 4a.b.c.and d) for training and ongoing oversight of the BCIO _____

3. Refer to BCIO approved training procedures _____

4. Records maintained for all BCIO oversight _____

5. 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, PAC, RAC, or PPAC and BCC _____

c. Report paired results (CFU/mL and IBC/mL) as specified by the FDA _____

APPARATUS

6. BactoCount IBC (BCC) Model _____

a. BCC 50 (speed 50 samples per hour) _____ _____

b. BCC 100 (speed 100 samples per hour) _____ _____

c. BCC 150 (speed 150 samples per hour) _____ _____

REAGENTS

7. Purified Water, deionized (conductivity less the 2 µS/cm, see CP item 24c3) _____

8. BactoCount Reagents supplied by manufacturer _____

a. Nucleic Acid Marker Lot #: _____ Exp. Date: _____ _____

b. Enzyme Lot #: _____ Exp. Date: _____ _____

c. Solubilizer Lot #: _____ Exp. Date: _____ _____

d. Lysing Buffer Powder Lot #: _____ Exp. Date: _____ _____

e. Staining Buffer Part 1 Lot #: _____ Exp. Date: _____ _____

f. Staining Buffer Part 2 Lot #: _____ Exp. Date: _____ _____

g. RBS Cleaning Concentrate Lot #: _____ Exp. Date: _____ _____

h. Microspheres Lot #: _____ Exp. Date: _____ _____

i. Triton X-100 Lot #: _____ Exp. Date: _____ _____

j. IBC Control Standard Lot #: _____ Exp. Date: _____ _____

9. Bentley Disposable Filter Unit for Liquids, 0.2 µm _____

10. All chemicals not provided by manufacturer, Analytical Grade _____

11. Stock Solutions _____

a. Buffer Stock Solution _____

1. Pour one bag of Lysing Buffer Powder (item 8d) into a container 10 L or larger _____
2. Add 10 L purified water (item 7) _____
3. Heat to 50°C and stir until completely dissolved _____
4. Add one 10 mL vial of Solubilizer (item 8c) _____
5. Mix until completely dissolved _____
6. Store for up to 6 months at room temperature _____

Lab Prep Date: _____ Exp. Date: _____ _____

b. Dye Stock Solution _____

1. Pour Staining Buffer Part 1 (item 8e) and Staining Buffer Part 2 (item 8f) into a 1 L container _____
2. Add 900 mL purified water (item 7) _____
3. Mix until completely dissolved. Do not heat _____
4. Add the Nucleic Acid Marker (item 8a), carefully rinsing all the contents of the vial into the solution with purified water (item 7) _____
5. Add purified water (item 7) up to the 1000 mL mark _____
6. Mix until completely dissolved. Do not heat _____
7. When not in use, store in the dark for up to 6 months in the refrigerator (0.0 - 4.5°C) _____

Lab Prep Date: _____ Exp. Date: _____ _____

c. Microsphere Stock Solution _____

1. Add one (1) drop of Microspheres (item 8h) to a 2 L container _____
2. Add 2 L purified water (item 7) _____
3. Add 20 mL Triton X-100 (item 8i) _____
4. Mix until completely dissolved. Do not heat _____

5. Store for up to 1 year in the refrigerator (0.0 - 4.5°C). Do not freeze _____

Lab Prep Date: _____ Exp. Date: _____ _____

12. Working Solutions _____

a. Incubation Reagent _____

1. Pour 1800 mL Buffer Stock Solution (item 11a), 100 mL Dye Stock Solution (item 11b) and 100 mL Enzyme (item 8b) into a 2 L container _____

2. Mix thoroughly _____

3. When not in use, store in refrigerator (0.0 - 4.5°C). Use within 7 days _____

Lab Prep Date: _____ Exp. Date: _____ _____

b. Carrier Fluid _____

1. Pour 400 mL RBS Cleaning Concentrate (item 8g) into a 20 L container _____

2. Add 19.6 L purified water (item 7) _____

3. Store at room temperature for up to 7 days- _____

Lab Prep Date: _____ Exp. Date: _____ _____

c. Microsphere Working Solution _____

1. Pour 20 mL Microsphere Stock Solution (item 11c) and 180 mL purified water (item 7) into a 200 mL container _____

2. Mix thoroughly _____

3. Store for up to 6 months in refrigerator (0.0 - 4.5°C). Do not freeze _____

Lab Prep Date: _____ Exp. Date: _____ _____

d. Rehydrated IBC Control Standard _____

1. Pour 60 mL Buffer Stock Solution (item 11a) into a container _____

2. Let the IBC Control Standard (item 8j) (V1) and the Buffer Stock Solution (item 11a) (V2) adjust to room temperature for 15 minutes _____

3. Using a disposable transfer pipette or pipet tip, transfer approximately 5 mL of fluid from V2 into V1. Let it dissolve for 2 minutes _____

4. Refill the pipette with clean fluid from V2 _____

5. Pour the contents of V1 into V2. Use the contents of the pipette to rinse out V1 into V2. Mix gently _____

6. Let the mixture dissolve in V2 for 10±1 minutes _____

7. Mix V2 gently _____

8. The rehydrated IBC Control Standard can be stored for up to 96 hours in the refrigerator (0.0 - 4.5°C) _____

Lab Prep Date: _____ Exp. Date: _____ _____

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

START-UP

14. Daily Instrument Start-up _____

a. Check the filters (item 9) in positions F1, F2, F3, and CFP _____

1. F1: changed within last 7 days and has no cracks _____

2. F2: changed within last 14 days and has no cracks _____

3. F3: changed within last 14 days and has no cracks _____

4. CFP: changed within the last month and has no cracks _____

5. If a filter is past its expiration date or is cracked, it must be replaced _____

6. All filters labeled with date installed or equivalent record _____

b. Confirm that the incubation reagent (item 12a) is within expiration date. If not, discard and make a fresh mix _____

c. At the end of the incubation reagent intake line, replace the bottle containing purified water (item 7) with a bottle containing incubation reagent (item 12a) _____

d. Confirm that the carrier fluid (item 12b) is within expiration date. If not, discard and make a fresh mix _____

e. Check the syringes and seals for leaks _____

1. Confirm that no moisture has gathered under syringes in positions P9, P10, P11, and P12 _____

2. If moisture is found under a syringe, replace the syringe, or alternatively replace the syringe seal _____

f. Switch the system on _____

As the instrument warms up

g. Prime the incubation reagent (minimum one (1) cycle) _____

h. Check the instrument zero by running water samples _____

1. Fill a container (min. 200 mL) with purified water (item 7) and set it under the sample intake pipette _____

2. Create a batch by clicking the ‘Batch’ icon, giving the bath a unique ID and choosing the Batch Type ‘Normal’. Make sure that the ‘Autosampler Rack Advance’ is disabled _____

3. For the BCC 50 (item 6a) run 15 water samples, for the BCC 100 (item 6b) and the BCC 150 (item 6c) run 33 water samples _____

4. Alternatively, fill 15 vials (for the BCC 50 (item 6a)) or 33 vials (for the BCC 100 (item 6b) and BCC 150 (item 6c)) with purified water (item 7) and place them in a rack _____

5. Create a batch by clicking the ‘Batch” icon, giving the batch a unique ID and choosing the Batch Type ‘Normal’. Make sure that the ‘Autosampler Rack Advance’ is enabled _____

6. Run the batch _____

i. When the water samples have been tested, confirm that the average count is <5 K IBC. If not, repeat item 14h until specification is met _____

j. Prepare and analyze the Microsphere Working Solution (item 12c) _____

1. Confirm that the Microsphere Working Solution (item 12c) is within expiration date. If not, discard and make a fresh mix _____

2. Place a small container of the Microsphere Working Solution (item 12c) on the carousel deck _____

3. Pull the cytometer line out of its holder and place it directly into the Microsphere Working Solution (item 12c) _____

4. Choose the ‘Microspheres’ Batch Type and run a ‘Microspheres’ batch with 10 samples _____

5. Place the cytometer line back in its holder _____

k. When the Microsphere Working Solution (item 12c) has been analyzed, confirm that the instrument is stable and aligned _____

1. STD < 0.015 (Log Unit) _____

2. Average Height Curve is bell shaped (Gaussian) _____
3. Average Height Curve is centered on the Recommended Intensity

Value (RIV) ± 0.1 _____

4. If above parameters are not met, adjust the alignment and/or the PCB/PMT gain factors and repeat item 14j until specifications are met _____

5. If laser alignment is performed and/or the PCB/PMT gain factors are changed, repeat items 14h and 14i _____

l. Prepare a 5-sample rack for the Startup (Carry-Over) test _____

1. One (1) vial of purified water (item 7) _____

2. One (1) vial low control (low count routine milk sample) _____

3. One (1) vial of purified water (item 7) _____

4. One (1) vial high control (IBC Control Standard (item 12d)) _____

5. One (1) vial of purified water (item 7) _____

m. Run a Startup test (Carry-Over) _____

1. Create a unique Batch ID _____

2. Set the number of Samples to five (5) _____

3. Choose Batch Type ‘Startup’ _____

4. Set the number of Repeats to five (5) _____

5. Make sure that the ‘Autosampler Rack Advance’ is enabled _____

6. Run the batch _____

n. After the Startup batch has been analyzed: _____

1. Confirm that IBC Control Standard (item 12d) is within spec: _____

a. High Control (Sample 4) = reference value (on COA) ± 10% _____

b. If not, discard the IBC Control Standard (item 12d) and repeat item 12d, item 14l, and item 14m _____

2. Confirm that the Standard Deviations (repeatability) and Carry-over levels are acceptable _____

a. Low control (Sample 2) < 0.060 STD LOG (IBC) _____

b. High control (Sample 4) < 0.060 STD LOG (IBC) _____

c. Carryover to Sample 5 < 1% _____

d. If the above parameters are not met, repeat steps 14l and 14m until specifications are met _____

o. If any of the parameters in items 14i, 14k, or 14n fall outside of specification and do not correct after re-measurement, seek technical assistance _____

p. Do not proceed with sample counting if any of the parameters in items 14i, 14k, or 14n fall outside of specifications _____

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

PROCEDURE

15. Handling Samples _____

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

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

16. 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. Test the rehydrated IBC Control Standard (item 12d) on an hourly basis. Must be within ±10% of the reference value on COA _____

d. Samples run on the BactoCount IBC may be immediately placed into a 37-42°C water bath to run for ESCC. Inhibitor testing must be completed before heating _____

e. Alternatively, refer to CP item 33.a.7.a.1 _____

17. Results _____

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

b. Using the calibration entered into the instrument, K IBC/mL is converted to K CFU/mL and both outputs are listed in the report _____

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

18. Records _____

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

19. End of Day Procedure _____

a. Replace the Incubation reagent with purified water _____

b. Prime the incubation reagent (minimum one (1) cycle) _____

c. For BCC 50 (item 6a): _____

1. Fill one (1) sample vial with carrier fluid (item 12b) and one (1) sample vial with purified water (item 7) _____

2. Place the sample vials in the rack (carrier fluid vial first) _____

3. Run a batch of 2 samples and 11 repeats using the routine automatic testing procedure _____

d. For BCC 100 (item 6b) and BCC 150 (item 6c): _____

1. Fill three (3) sample vials with carrier fluid (item 12b) and three (3) sample vials with purified water (item 7) _____

2. Place the sample vials in the rack (carrier fluid vials first) _____

3. Run a batch of 6 samples and 11 repeats using the routine automatic testing procedure _____

e. Switch the system off _____

20. Reporting _____

a. Report the bacterial content of the milk as BCC CFU/mL (K CFU/mL x 1000 = CFU/mL) _____

1. Instrument reports in K CFU/mL, 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) _____