BENTLEY BACTOCOUNT IBCm
(Raw Commingled Cow Milk Only)
IMS #7d

[Unless otherwise stated all tolerances are ±5%]

GENERAL REQUIREMENTS

1. Cultural Procedures, 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 IBCm (BCMC) 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 IBCm Industry Operator (BCMIO) can analyze samples for regulatory compliance _____

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

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

3. Refer to BCMIO approved training procedures _____

4. Records maintained for all BCMIO 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 BCMC _____

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

APPARATUS

6. BactoCount IBCm (BCMC) Model _____

a. BCMC IBCm _____

b. BCMC Incubator _____

c. BCMC Sonicator _____

d. BCMC Sonicator rest _____

e. BCMC Stainless steel vials _____

f. BCMC Carrier fluid container _____

REAGENTS

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

8. BactoCount IBCm Reagents supplied by manufacturer _____

a. IBCm Bacto Kit Component 1 Lot #: _____ Exp. Date: _____ _____

b. IBCm Bacto Kit Component 2 Lot #: _____ Exp. Date: _____ _____

c. IBCm Bacto Kit Component 3 Lot #: _____ Exp. Date: _____ _____

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

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

f. Triton X-100, bottle Lot #: _____ Exp. Date: _____ _____

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

h. IBC Control Standard Buffer Solution Lot #: _____ Exp. Date: _____ _____

9. Other Consumables and Equipment Provided by Manufacturer _____

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

b. Syringe filter, 0.2 µm _____

10. Other Consumables and Equipment (Provided by Manufacturer or Equivalent) _____

a. Amber glass media bottle, 500 mL _____

b. Bottle top dispenser, 2 mL _____

c. Fixed volume pipette, 1 mL _____

d. Pipette tips, 100 - 1,000 µL _____

11. All chemicals not Provided by Manufacturer, Analytical Grade _____

12. Stock Solution _____

a. Microsphere Stock Solution _____

1. Add one (1) drop of Microspheres (item 8e) to a 2 L container _____

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

3. Add 20 mL Triton X-100 (item 8f) _____

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: _____ _____

13. Working Solutions _____

a. Incubation Reagent _____

1. Pour 18 parts IBCm Bacto Kit Component 1 (item 8a), 1 part IBCm Bacto Kit Component 2 (item 8b), and 1 part IBCm Bacto Kit Component 3 (item 8c) into a suitable container _____

2. Mix thoroughly _____

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

Lab Prep Date: _____ Exp. Date: _____ _____

b. Carrier Fluid _____

1. Pour 80 mL RBS Cleaning Concentrate (item 8d) into the Carrier Fluid Container (item 6f) _____

2. Add 3.92 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 12a) 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 IBC Control Standard Buffer Solution (item 8h) into a container _____

2. Let the IBC Control Standard (item 8g) (V1) and the IBC Control Standard Buffer Solution (item 8h) (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: _____ _____

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

START-UP

15. Daily Instrument Start-up _____

a. Check the Bentley filter (item 9a) on top of the carrier fluid pump (position P3) _____

1. On top of P3: changed within the last month and has no cracks. If filter is past its expiration date or is cracked, it must be replaced. Filter labeled date installed or equivalent record _____

b. Confirm that the carrier fluid (item 13b) is within expiration date. If not, discard and make a fresh mix _____

c. Check the syringes and seals for leaks _____

1. Confirm that no moisture has gathered under syringes and seals _____

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

d. Switch the system on. Wait for the instrument to initialize _____

e. Confirm that the incubator (item 6b) is on and heating _____

1. Indicator lights on the incubator (item 6b) will be blinking _____

f. Start the software. Wait for the system to initialize _____

g. Confirm that the incubator (item 6b) is connected _____

1. Indicator lights on the incubator (item 6b) will stop blinking and be on _____

h. Place a small beaker with purified water (item 7) under the sample intake pipette. Start running samples under the Microsphere setting to eliminate air pockets from the system _____

i. Total warm-up time is 30 minutes _____

As the instrument warms up

j. Fill a beaker with a minimum of 500 mL of carrier fluid (item 13b) for vial cleaning _____

k. Fill a beaker with a minimum of 500 mL of purified water (item 7) for vial rinsing _____

l. Clean stainless steel vials (item 6e) in the cleaning solution (item 15j), then rinse in purified water (item 15k), briefly place then bottom up on an absorbent material and then place them bottom down on the preheating area of the incubator (item 6b) _____

m. Confirm that the incubation reagent (item 13a) is within expiration date. If not, discard and make a fresh mix _____

n. Pour incubation reagent (item 13a) into amber glass media bottle (item 10a) and affix the bottle top dispenser (item 10b). Pump 2 - 3 strokes to expel possible air pockets, apply fresh syringe filter (item 9b), pump another 2 - 3 strokes to prime the filter _____

o. Confirm that the Microsphere Working Solution (item 13c) is within expiration date. If not, discard and make a fresh mix. _____

When the instrument is warmed up

p. Check the instrument zero by testing purified water (item 7) samples _____

1. Test a total of five (5) purified water (item 7) samples using the routine testing procedure (item 17) _____

2. After testing is completed, confirm that the average count is <5K IBC. If not, repeat item 15p1 until specification is met. _____

q. Analyze the Microsphere Working Solution (item 13c) _____

1. Place a small container of the Microsphere Working Solution (item 13c) under the sample intake pipette _____

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

3. When the Microsphere Working Solution (item 13c) has been analyzed, confirm that the instrument is stable and aligned _____

a. STD < 0.015 (Log Unit) _____

b. Average Height Curve is bell shaped (Gaussian) _____

c. 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 15q until specifications are met _____

5. If laser alignment is performed and/or the PCB/PMT gain factors are changed, repeat item 15p _____

r. Check Instrument and chemical functionality by testing the rehydrated IBC Control Standard (item 13d) _____

1. Test five (5) IBC Control Standard (item 13d) samples using the routine testing procedure (item 17) _____

2. After testing is completed, confirm that results are within specifications _____

   a. Average Height Curve is bell shaped (Gaussian) _____

   b. The average count is within ±10% of the reference value found on the Certificate of Analysis _____

3. If the above parameters are not met, repeat item 15r until specifications are met _____

s. If any of the parameters in items 15p2, 15q3, or 15r2 fall outside of specification and do not correct after re-measurement, seek technical assistance _____

t. Do not proceed with sample counting if any of the parameters in items 15p2, 15q3, or 15r2 fall outside of specifications _____

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

PROCEDURE

16. Handling Samples _____

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

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

17. Testing Samples _____

a. Test the rehydrated IBC Control Standard (item 14d) hourly if official testing is done within that hour. Must be within ±10% of the reference value on COA _____

b. Before testing the samples, invert them 10 times to mix _____

c. Add 2.0 mL incubation reagent (item 13a) to a preheated stainless steel vial (item 6e), using the supplied bottle top dispenser (item 10b) _____

d. Transfer 1.0 mL of the sample to the stainless steel (item 6e), using the supplied fixed volume pipette (item 10c) and pipette tips (item 10d) _____

e. Place the filled stainless steel vial (item 6e) on one of the designated incubation slots on the incubator (item 6b) _____

f. Double Click on vial image on screen _____

g. Enter Sample Identification and Choose Product: Cow IBC in pop up window _____

h. At preset times during the incubation the software will request a round of sonication. Place the sonicator (item 6c) on top of the stainless steel vial (item 6e), push downwards and release promptly. The sonicator will be activated for the required time. When sonication is completed, place the sonicator back in the sonicator rest (item 6d) _____

i. When incubation time is completed, immediately (no longer than 30 seconds) move the vial to the area under the sample intake pipette _____

j. Using the software, start the sample. The sample intake pipette will pull the sample automatically and the counting starts _____

k. When the sample has been pulled, discard the remaining liquid _____

l. Clean the stainless steel vial (item 6e) in the cleaning solution (item 15j), then rinse in the purified water (item 15k), briefly place the vial bottom up on and absorbent material and the place it bottom down on the preheating area of the incubator (item 6b) _____

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

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

18. 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 _____

19. Records _____

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

20. End of Day Procedure _____

a. Replace the container with carrier fluid (item 13b) under the sample intake pipette _____

b. Run 10 samples under the ‘Microsphere’ setting _____

c. Place a container with purified water (item 7) under the sample intake pipette _____

d. Run 10 samples under the ‘Microsphere’ setting _____

e. Switch the system off _____

21. Reporting _____

a. Report the bacterial content of the milk as BCMC 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) _____