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

[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°C). 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)** ________
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)