

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

[Unless otherwise stated all tolerances are  $\pm 5\%$ ]

**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  $\mu$ S/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  $\mu$ m** \_\_\_\_\_

**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)  $\pm$  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

\_\_\_\_\_

i. 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)  $\pm$  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 ( $K \text{ CFU/mL} \times 1000 = \text{CFU/mL}$ )
  - 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)