BENTLEY BACTOCOUNT IBC INDUSTRY OPERATOR (BCIO) APPROVAL PROCEDURES PROTOCOL

A. Training:

1. BactoCount IBC Industry Operators (BCIO) are to receive one week of training conducted by a certified BactoCount IBC analyst.

2. Follow the most current approved BactoCount IBC 2400 Form requirements for training and testing.

3. Training Log signed by certified BactoCount IBC analyst and BCIO.

4. Records maintained.

B. Daily Instrument Start Up Procedure:

1. Check the filters in positions F1, F2, F3 and CFP:

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

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

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

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

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

    f. All filters labeled with date installed or equivalent record.

2. Confirm that the incubation reagent is within expiration date. If not, discard and make a fresh mix:

    a. Pour 1800 mL Buffer Stock Solution, 100 mL Dye Stock Solution, and 100 mL Enzyme into a 2 L container. Mix thoroughly.

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

    c. Label container with Date Prepared and Expiration Date.

3. At the end of the incubation reagent intake line, replace the bottle containing purified water with a bottle containing incubation reagent.

4. Confirm that the carrier fluid is within expiration date. If not, discard and make a fresh mix:

    a. Pour 400 mL RBS Cleaning Concentrate into a 20 L container.

    b. Add 19.6 L purified water.

c. Store at room temperature up to 7 days.

d. Label container with Date Prepared and Expiration Date.

5. Check the syringes and seals for leaks:

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

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

6. Switch the system on.

C. As the instrument warms up:

1. Confirm that the Microsphere Working Solution is within expiration date. If not, discard and make a fresh mix:

a. Pour 20 mL Microsphere Stock Solution and 180 mL purified water into a 200 mL container. Mix thoroughly.

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

c. Label container with Date Prepared and Expiration Date.

2. Confirm that the rehydrated IBC Control Standard is within expiration date. If not, discard and make a fresh mix:

a. Pour 60 mL Buffer Stock Solution into a container.

b. Let the IBC Control Standard (V1) and the Buffer Stock Solution (V2) adjust to room temperature for 15 minutes.

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

d. Refill the pipette with clean fluid from V2.

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

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

g. Mix V2 gently.

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

i. Label container with Date Prepared and Expiration Date.

D. When Instrument is warmed up:

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

2. Check the instrument zero by running water samples:

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

    b. 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 disabled.

    c. For the BCC 50 run 15 water samples, for the BCC 100 and BCC 150 run 33 water samples.

    d. Alternatively, fill 15 vials (for the BCC 50) or 33 vials (for the BCC 100 and BCC 150) with purified water and place them in a rack.

    e. 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.

    f. Run the batch.

3. When the water samples have been tested, confirm that the average count is <5 K IBC. If not, repeat step D2 until specification is met.

4. Analyze the Microsphere Working Solution:

    a. Place a small container of the Microsphere Working Solution on the carousel deck.

    b. Pull the cytometer line out of its holder and place it directly into the Microsphere Working Solution.

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

    d. Place the cytometer line back in its holder.

5. When the Microsphere Working Solution 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

    d. If the above parameters are not met, adjust the alignment and/or the PCB/PMT gain factors and repeat step D4 until specifications are met

    e. If laser alignment is performed and/or the PCB/PMT gain factors are changed, repeat steps D2 and D3

6. Prepare a 5-sample rack for the Startup (Carry-Over) test:
    a. One (1) vial purified water.
    b. One (1) vial low control (low count routine milk sample).
    c. One (1) vial purified water.
    d. One (1) vial high control (IBC Control Standard).
    e. One (1) vial purified water.
7. Run a Startup test (Carry-Over):
    a. Create a unique Batch ID.
    b. Set the number of Samples to five (5).
    c. Choose Batch Type ‘Startup’.
    d. Set the Number of Repeats to five (5).
    e. Make sure that the ‘Autosampler Rack Advance’ is enabled.
    f. Run the batch.
8. After the Startup batch has been analyzed:
    a. Confirm that the IBC Control Standard (step D6d) is within spec:
        1. High control (Sample 4) = reference value (on COA) ± 10%.
        2. If not, discard and remake the IBC Control Standard (See step C2) and repeat steps D6, D7, and D8.
    b. Confirm that the Standard Deviations (repeatability) and Carry-over levels are acceptable:
        1. Low control (Sample 2) < 0.060 STD LOG (IBC).
        2. High control (Sample 4) < 0.060 STD LOG (IBC).
        3. Carryover to Sample 5 < 1%.
        4. If the above parameters are not met, repeat steps D6 and D7 until specifications are met.
9. If any of the parameters in steps D3, D5, or D8 fall outside of specifications and do not correct after re-measurement, seek technical assistance.
10. Do not proceed with sample counting if any of the parameters in steps D3, D5, or D8 fall outside of specifications.
11. Records to be maintained on all parameters each time the instrument is used.

E. Handling Samples:

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

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

F. Testing Samples:

1. Before placing the samples in the racks, invert them 10 times to mix, or place samples in rack and invert rack with samples 10 times to mix.

2. Place rack on conveyor and start the automatic testing procedure immediately.

3. Test the rehydrated IBC Control Standard (step C2) on an hourly basis. Must be within ±10% of the reference value on COA.

4. 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.

G. Results:

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

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

H. Records:

1. Maintain records of all results, controls and samples.

2. All records signed by a certified BactoCount IBC analyst.

I. End of Day Procedure:

1. Replace the Incubation Reagent with purified water.

2. Prime the incubation reagent (minimum one (1) cycle).

3. For BCC 50:

    a. Fill one (1) sample vial with carrier fluid and one (1) sample vial with purified water.

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

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

4. For BCC 100 and BCC 150:

    a. Fill three (3) sample vials with carrier fluid and three (3) sample vials with purified water.

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

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

5. Switch the system off.

J. Proficiency (Initial Approval then Monthly):

1. Have BCIO analyze one set of 10 split milk samples.

2. Then have certified analyst analyze the other replicate set of 10 split milk samples.

3. Compare test results against each other to ensure results are comparable.

4. Records maintained.

K. Evaluation (Monthly):

1. Spot check BCIO performing different areas of the operation (e.g. start-up, making rehydrated IBC Control Standard, check prep dates, shut downs, records, etc.).

2. Records maintained.

A BCIO can run official samples for regulatory purposes without a certified BactoCount IBC analyst on site or present, but available to the BClO operator.