

**BENTLEY BACTOCOUNT IBCm INDUSTRY OPERATOR (BCMIO)
APPROVAL PROCEDURES PROTOCOL**

A. Training:

1. BactoCount IBCm Industry Operators (BCMIO) are to receive one week of training conducted by a certified BactoCount IBCm analyst.
2. Follow the most current approved BactoCount IBCm 2400 Form requirements for training and testing.
3. Training Log signed by certified BactoCount IBCm analyst and BCMIO.
4. Records maintained.

B. Daily Instrument Start Up Procedure:

1. Check the Bentley filter on top of the carrier fluid pump (position P3):
 - a. 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 with date installed or equivalent record.
2. Confirm that the carrier fluid is within expiration date. If not, discard and make a fresh mix:
 - a. Pour 80 mL RBS Cleaning Concentrate into the Carrier Fluid Container. Add 3.92 L purified water.
 - b. Store at room temperature up to 7 days.
 - c. Label container with Date Prepared and Expiration Date.
3. Check the syringes and seals for leaks:
 - a. Confirm that no moisture has gathered under syringes and seals.
 - b. If moisture is found under a syringe, replace the syringe, or alternatively, replace the syringe seal.
4. Switch the system on. Wait for the instrument to initialize.
5. Confirm that the incubator is on and heating:
 - a. Indicator lights on the incubator will be blinking.
6. Start the software. Wait for the system to initialize.
7. Confirm that the incubator is connected:
 - a. Indicator lights on the incubator will stop blinking and be on.

8. Place a small beaker with purified water under the sample intake pipette. Start running samples under the Microsphere setting to eliminate air pockets from the system.
9. Total warm-up time is 30 minutes.

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.
3. Fill a beaker with a minimum of 500 mL of carrier fluid for vial cleaning.
4. Fill a beaker with a minimum of 500 mL of purified water for vial rinsing.
5. Clean the stainless steel vials in the cleaning solution, then rinse in purified water, briefly place them bottom up on an absorbent material and then place them bottom down on the preheating area of the incubator.

6. Confirm that the incubation reagent is within expiration date. If not, discard and make a fresh mix:
 - a. Pour 18 parts IBCm Bacto Kit Component 1, 1 part IBCm Bacto Kit Component 2, and 1 part IBCm Bacto Kit Component 3 into a suitable 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.
7. Pour incubation reagent into amber glass media bottle and affix the bottle top dispenser. Pump 2-3 strokes to expel possible air pockets, apply fresh syringe filter, pump another 2-3 strokes to prime the filter.

D. When instrument is warmed up:

1. Check the instrument zero by testing purified water samples:
 - a. Test a total of five (5) purified water samples using the routine testing procedure.
 - b. After testing is completed, confirm that the average count is <5 K IBC. If not, repeat step D1a until specification is met.
2. Analyze the Microsphere Working Solution:
 - a. Place a small container of the Microsphere Working Solution under the sample intake pipette.
 - b. Choose the 'Microspheres' Batch Type and run a 'Microspheres' batch with 10 samples.
 - c. When the Microsphere Working Solution 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 .
 - d. If above parameters are not met, adjust the alignment and/or the PCB/PMT gain factors and repeat step D2 until specifications are met.
 - e. If laser alignment is performed and/or the PCB/PMT gain factors are changed, repeat step D1.

3. Check instrument and chemical functionality by testing the rehydrated IBC Control Standard:
 - a. Test a total of five (5) IBC Control Standard samples using the routine testing procedure.
 - b. After testing is completed, confirm that results are within specifications:
 1. Average Height Curve is bell shaped (Gaussian).
 2. The average count is within $\pm 10\%$ of the reference value found on the Certificate of Analysis.
 - c. If the above parameters are not met, repeat step D3 until specifications are met.
4. If any of the parameters in steps D1, D2 or D3 fall outside of specifications and do not correct after re-measurement, seek technical assistance.
5. Do not proceed with sample counting if any of the parameters in D1, D2 or D3 fall outside of specifications.
6. 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 IBCm.
2. Samples must be kept at 0.0-4.5°C until tested.

F. Testing Samples:

1. Test the rehydrated IBC Control Standard hourly if official testing is done within that hour. Must be within $\pm 10\%$ of the reference value on COA.
2. Before testing the samples, invert them 10 times to mix.
3. Add 2.0 mL incubation reagent to a preheated stainless steel vial, using the supplied bottle top dispenser.
4. Transfer 1.0 mL of the sample to the stainless steel vial using the supplied fixed volume pipette and pipette tips.
5. Place the filled stainless steel vial on one of the designated incubation slots on the incubator.
6. Double Click on vial image on screen.
7. Enter Sample Identification and Choose Product: Cow IBC in pop up window.

8. At preset times during incubation, the software will request a round of sonication. Place the sonicator on top of the stainless steel vial, 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.
9. When incubation time is completed, move the vial to the area under the sample intake pipette.
10. Using the software, start the sample. The sample intake pipette will pull the sample automatically and the counting starts.
11. When the sample has been pulled, discard the remaining liquid.
12. Clean the stainless steel vial in the cleaning solution, then rinse in the purified water, briefly place the vial bottom up on an absorbent material and then place it bottom down on the preheating area of the incubator.
13. 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.
14. Alternatively, refer to CP item 33.a.7.a.1.

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 IBCm analyst.

I. End of Day Procedure:

1. Place a container with carrier fluid under the sample intake pipette.
2. Run 10 samples under the 'Microsphere' setting.
3. Place a container with purified water under the sample intake pipette.
4. Run 10 samples under the 'Microsphere' setting.
5. Switch the system off.

J. Proficiency (Initial Approval then Monthly):

1. Have BCMIO 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 BCMIO 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 BCMIO can run official samples for regulatory purposes without a certified BactoCount IBCm analyst on site or present, but available to the BCMIO operator.