

**BACTO-SCAN INDUSTRY OPERATOR (BIO)
APPROVAL PROCEDURES PROTOCOL**

A. Training:

1. BactoScan Industry Operators (BIO) are to receive two weeks of training conducted by a certified BactoScan analyst.
2. Follow the most current approved BactoScan 2400 Form requirements for training and testing.
3. Training Log signed by certified BactoScan analyst and BIO.
4. Records maintained.

B. Daily Instrument Start Up Procedure:

1. Replace Used Incubation Reagent Filter on Intake Assembly
 - a. Lift spring loaded disc that holds the filter in position.
 - b. Remove and discard the old filter
 - c. Insert the new filter and release disc.
2. Prepare Incubation Reagent
 - a. For 150 samples/hour with 8 hour run time: Add 1600 mL ($\pm 2\%$) of Staining Reagent and 1 bottle of Enzyme 150 to Incubation Reagent container. Invert container 10 times to mix before use.
 - b. For 100 samples/hour with 8 hour run time: Add 1100 mL ($\pm 2\%$) of Staining Reagent and 2 bottles of Enzyme 50 to Incubation Reagent container. Invert container 10 times to mix before use.
 - c. For 50 samples/hour with 8 hour run time: Add 550 mL ($\pm 2\%$) of Staining Reagent and 1 bottle of Enzyme 50 to Incubation Reagent container. Invert container 10 times to mix before use.
 - d. Must be used on day of preparation. Discard any left over.
 - e. Label container with Date Prepared.
3. Prepare Sheath Reagent (Ready To Use).
 - a. Using a 10 L container, pour in 8 L ($\pm 10\%$) of purified de-ionized water, and then add 2 L ($\pm 10\%$) of Sheath Liquid Stock Solution.
 - b. Replace lid and invert container 10 times to mix before use.

- c. Store at room temperature (< 25C) up to 7 days or at 25-35C up to 2 days.
- d. Label container with Date Prepared and Expiration Date.
 - 1. Check expiration date daily. Sheath Reagent must be replaced when expired.
- 4. Check Large Rinse Solution Container.
 - a. Pour 100 mL of Rinse Concentrate into the 50 L container.
 - b. Then add 50 L of purified de-ionized water to ensure complete mixing of the two liquids.
 - c. For daily fill ups, pour 20 mL of Rinse Concentrate into 10 L container. Then add 10 L of purified de-ionized water to ensure complete mixing of the two liquids.
 - d. Label container with Date Prepared and Expiration Date.
 - e. Rinse solution must be replaced discarded every 7 days.
- 5. Prepare Blank Solution
 - a. Mix 1 L (\pm 10%) of purified de-ionized water and 50 mL (\pm 10%) of Sheath Liquid Stock Solution in a sterilized 1 L container.
 - b. Invert 10 times to mix before use.
 - c. Must be used on day of preparation. Discard any left over.
 - d. Label container with Date Prepared.
- 6. Prepare End of Day Solution (Ready to Use).
 - a. Pour 10 L (\pm 10%) of purified de-ionized water and add 50 mL (\pm 10%) of ammonia (25% analytical grade).
 - b. Invert 10 times to mix well.
 - c. Can be stored at room temperature (< 25C) for a maximum of 7 days (discard left over solution and make up fresh solution).
 - d. Label container with Date Prepared and Expiration Date.
- 7. Transfer probes from the End of Day Solution Container.
 - a. Transfer the Rinse and Incubation Reagent probes from End of Day Solution container to appropriate liquid containers.

- b. Place Rinse Solution probe into the 50 L Rinse Solution container and Incubation Reagent probe into the Incubation Reagent Solution container.
8. Turn BactoScan System on.

C. As Instrument Warms Up:

1. Prepare Bacterial Control Sample (BCS)
 - a. Using a 100 mL graduated cylinder, measure 100 mL ($\pm 2\%$) of Rehydration Solution to a suitable container with lid.
 - b. Take a BCS vial from the freezer.
 1. Remove metal cap seal and loosen the lid.
 2. Using a sterile disposable 5 mL pipette, transfer 2-3 mL of Rehydration Solution into the BCS vial.
 3. Close the BCS vial and shake to completely dissolve.
 4. Refill the 5 mL pipette with clean Rehydration Solution.
 5. Pour the dissolved BCS vial contents into the Rehydration Solution container.
 6. Use the contents of the refilled 5 mL pipette to rinse the BCS vial and pour the rinse contents into the Rehydration Solution container.
 7. Close lid and shake well for complete mixing.
 8. Store the reconstituted BCS container in the refrigerator, 0-4.4C.
 9. The reconstituted BCS can be stored for up to 10 hours when kept at 0-4.4C.
 10. Label reconstituted BCS container with Date and Time Prepared.
2. Prepare Raft or Float with Control Sample Batch Rack (Hourly's).
 - a. Place 9 vials in the float or raft.
 1. Vials 1-4, fill with Blank Solution.
 2. Vial 5, label "BCS" and fill with Re-constituted Bacterial Control Solution.
 3. Vials 6-9, fill with Blank Solution.
 - b. Store the control samples in refrigerator at 0-4.4C when not in use.

3. Run a Control Sample Batch Rack.
 - a. Enter appropriate batch type into the system (e.g., start-up) to ensure the correct presentation and calculation of results.
 - b. Check BCS lot number to see that it corresponds with the lot being used.
 - c. Measure the Control Sample Batch Rack
 1. Measure the control samples at the start and end of each sample testing run.
 2. Additionally, control samples must be measured every 60 minutes, maximum, throughout the working day.
 3. Records maintained.
4. When the Control Sample Batch Rack has been measured
 - a. Check that the Blank Solution counts are within acceptable limits (results of vials 2-4 and 7-9 no higher than 1 CFU).
 - b. Check that the BCS results conform to the limits specified for the lot in use (vial 5).
 - c. If BCS sample or Blank Solution counts are outside the limits and do not correct after re-measurement, STOP and call a certified BactoScan analyst and/or seek technical assistance. **Do not proceed until corrected.** Records of corrective actions taken maintained.
 - d. The control samples can be re-used up to 10 hours with acceptable results when maintained at 0-4.4C.
 - e. Records are to be maintained on all parameters each time instrument is used.

D. Sample Handling:

1. Samples must first be tested for presence of inhibitor before testing on the BactoScan.
2. Samples kept at 0-4C until placed in racks for testing.
3. Invert samples 10 times to mix before placing into testing racks.

E. Testing Samples:

1. Enter identifying batch information into system (e.g., type, number of samples, etc.).
2. Place inverted samples into testing rack, place rack on conveyor and immediately start the automatic testing procedure.
3. Samples run on BactoScan may be immediately placed into a 37-42C water bath to be tested for somatic cells.

F. Results:

1. The BactoScan read out is in IBC (Individual Bacteria Counts) / μ L, which is converted to CFU (Colony Forming Units) / μ L automatically by the BactoScan and printed out on the BactoScan reports.

G. Records:

1. Maintain records on all results, controls and samples daily.
2. All records signed by a certified BactoScan analyst.

H. End of Day Shut Down & Cleaning:

1. Place the probes for Incubation Reagent and Rinse Solution into the End of Day Solution container. Leave the Sheath Liquid probe in the Sheath Liquid Solution container.
2. Start the automatic cleaning procedure, a 20 min cycle.
3. Shut computer system down.

I. Proficiency (Initial Approval then Monthly):

1. Have BIO 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

J. Evaluation (Monthly):

1. Spot check BIO performing different areas of the operation (e.g. start-up, making BCS, check prep dates, shut downs, records, etc.).
 2. Records maintained.
- A BIO can run official samples for regulatory purposes without a certified BactoScan analyst on site or present, but available to the BIO operator.