FOSSOMATIC BACSOMATIC™

(Raw Commingled Cow Milk)
IMS # 7 & 16

(Unless otherwise stated all tolerances are ±5%)

GENERAL REQUIREMENTS

1. Cultural Procedures (CP) items 1-32, as appropriate _____

2. Sample Requirements, see CP items 33 & 34 _____

a. Raw milk tested only _____

b. Bacteria counting: Un-preserved samples may be run up to 60 hours after initial collection _____

c. SCC Counting _____

1. Un-preserved samples may be run up to 72 hours after initial collection _____

2. Samples may be tested up to 7 days after initial collection if preserved with 0.02% 2-bromo-2-nitropropane- 1,3-diol (Bronopol™) or 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

d. Bacteria and SCC counting (simultaneous) _____
[Criteria for bacteria counting (item 2.b) apply]

PRE-REQUISITES

3. Comparative Test with Standard Plate Count with Approved Media _____

a. Test 25 samples in duplicate using the SPC (2400a) or PAC (2400a-4) and BacSomatic method _____

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 comparisons and results in QC records (or easily accessible file in laboratory) _____

d. Analysts certified for SPC or PAC methods _____

4. Comparative Test with DMSCC _____
[NOT required as a co-requisite for certification of analysts in laboratories purchasing standards from a CERTIFIED provider (item 13.b)]

a. Analyst(s) certified for DMSCC _____

b. Each analyst seeking certification for the ESCC test shall perform the comparative test _____

1. Test 4 samples (100K-200K, 300K-500K, 600K-800K and 900K-1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC _____

2. Results must be evaluated by the FDA/LPET LEO or LEO and shown to be acceptable prior to official use of test in laboratory _____

3. Copy of comparison and results in QC record (or easily accessible on file in the laboratory); kept for as long as analyst is certified _____

APPARATUS

5. BacSomatic _____

a. Bacteria Counting ___ _____

b. SCC Counting ___ _____

REAGENTS

6. Purified Water, deionized (conductivity less than 2µS/cm, see CP item 24.c.3) and filter sterilized with a 0.2 µm filter _____

7. Reagents _____

a. BacSomatic SCC Dye _____

Lot #: _____ Exp. Date: _____ _____

b. BacSomatic IBC Kit _____

Lot #: _____ Exp. Date: _____ _____

c. BacSomatic Rinse Concentrate _____

Lot #: _____ Exp. Date: _____ _____

d. Bacterial Control Sample (BCS) _____

Lot #: _____ Exp. Date: _____ _____

e. Particle Control Sample (PCS) _____

Lot #: _____ Exp. Date: _____ _____

8. All Chemicals not Provided by Manufacturer, Analytical Grade _____

9. Stock Solutions _____

a. Preservation Stock Solution for Bacterial Control Sample (item 7.d) _____

1. Add 53 g Boric Acid, 0.8 g Potassium Sorbate and 10 g Glycerol into a 2 L container _____

2. Fill up to the 2000 mL mark with purified water (item 6) _____

3. Stir on stir plate until completely dissolved, to speed up process, heat to 40°C while stirring _____

4. Store at room temperature (< 25°C) for up to 10 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____ _____

b. Re-hydration Solution for Bacterial Control Sample (item 7.d) _____

1. Add one Ringer Tablet (Code BR 52) into a 1 L container _____

2. Add 300 mL purified water (item 6) and 200 mL Preservation Stock Solution (see item 9.a) _____

3. Stir until completely dissolved using stir plate _____

4. Store at room temperature (< 25°C) for up to 7 days _____

Lab Prep Date: _____ Lab Exp. Date: _____ _____

10. Ready to use Solutions _____

a. Blank solution: Purified Water (item 6) _____

b. Bacterial Control Sample (BCS) (item 7.d) _____

1. Bacterial Control Sample option 1 (must be used within 1 hour) _____

a. Measure 100 mL (± 2%) of purified water (item 6) and transfer it to a suitable container with a lid _____

b. Take a Bacterial Control Sample vial (item 7.d) from the freezer _____

1. Remove the metal cap and loosen the lid _____

2. Use a small transfer pipette to transfer 2-3 mL of the purified water (item 6) into the vial _____

3. Close the vial and shake to completely dissolve _____

4. Refill the pipette with purified water (item 6) _____

c. When the Bacterial Control Sample is dissolved, pour the contents of the vial into the container (item 10.b.1.a) _____

1. Use the contents of the pipette (item 10.b.1.b.4) to rinse the vial _____

2. Pour the contents of the vial into the container with the dissolved Bacterial Control Sample _____

d. Put on the lid and shake well _____

e. Store in a refrigerator (0.0-4.5°C) except when filling sample vials _____

f. The re-constituted, preserved Bacterial Control Sample can be stored for up to 1 hour when kept in the refrigerator (0.0-4.5°C) _____

Lab Prep Date: _____ Lab Exp. Date: _____ _____

2. Bacterial Control Sample option 2 (can be stored and must be used within 10 hr), Ready to Use Bacterial Control Sample (BCS) (item 7.d) _____

a. Measure 100 mL (± 2%) of Re-hydration Solution (item 9.b) and transfer it to a suitable container with a lid _____

b. Take a Bacterial Control Sample vial (item 7.d) from the freezer _____

1. Remove the metal cap and loosen the lid _____

2. Use a small sterile, disposable 5 mL pipette to transfer 2-3 mL of the Re-hydration Solution (item 9.b) into the vial _____

3. Close the vial and shake to completely dissolve _____

4. Refill the pipette and clean Re-hydration Solution (item 9.b) _____

c. When the Bacterial Control Sample is dissolved, pour the contents of the vial into the container (item 10.b.2.a) _____

1. Use the contents of the pipette (item 10.b.2.b.4) to rinse the vial _____

2. Pour the contents of the vial into the container with the dissolved Control Sample _____

d. Put the lid on and shake well _____

e. Store in a refrigerator (0.0-4.5°C) except when filling sample vials _____

f. The re-constituted, preserved Bacterial Control Sample can be stored for up to 10 hours when kept in the refrigerator (0.0-4.5°C) _____

Lab Prep Date: _____ Lab Exp. Date: _____ _____

c. End of Day Solution _____

1. Mix 500 mL of purified water (item 6) with 2.5 mL of 25% ammonia according to manufacturer's User Manual _____

11. Preparation of Reagents for the BacSomatic _____

a. Rinse/sheath liquid: Mix one bag of BacSomatic Rinse Concentrate (10 mL) with 5 L of purified water (item 6), store and use within 1 week _____

Lab Prep Date: _____ Lab Exp. Date: _____ _____

b. Insert BacSomatic SCC Dye bag according to manufacturer's instructions, use within 3 months _____

Insert Date: _____ Lab Exp. Date: _____ _____

c. Insert BacSomatic IBC kit (i.e. IBC Dye bag, Enzyme bag) (item 7.b) according to manufacturer's instructions, use within 3 months _____

Insert Date: _____ Lab Exp. Date: _____ _____

12. All solutions labeled with solution name, date prepared and expiration date, when relevant _____

13. Milk Standards _____

a. Commercially prepared: _____ _____

Lot#: _____ Date Rcd: _____ _____

1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

2. Perform DMSCC in triplicate on each standard in set and average counts; maintain records _____

3. Perform DMSCC check in rotation by all certified analysts _____

4. Standards used within one week _____

Lab Exp. Date: _____ _____

b. Certified provider: _____ _____

Lot #: _____ Exp. Date: _____ _____

Date Rcd: _____ _____

1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

2. Maintain copies of all provided DMSCC values _____

3. Measure and maintain records of temperature (0.0-7.5°C) of standards as received _____

4. Maintain copies of all correspondence regarding problems _____

5. Standards used by manufacturer's expiration date _____

6. Failed standards shall be verified with DMSCC _____

   a. If no analysts certified for DMSCC then a new set of standards is required _____

   b. Do not continue with official testing until the new standard(s) test(s) in range _____

c. Laboratory prepared (weekly) _____

1. Prepare from raw milk > 18 hours old preserved with 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

2. Or, preserved with 0.02% 2-bromo-2-nitropropane- 1,3-diol (Bronopol™) _____

3. Standards **cannot** be preserved with formalin _____

4. Prepare 4 standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M; use within one week _____

   Lab Prep Date: _____ Lab Exp. Date: _____ _____

5. Perform DMSCC in triplicate on each standard and average counts; maintain records _____

6. Perform DMSCC check in rotation by all certified analysts _____

d. Hourly Control Sample (instrument drift check) _____

1. Use one of the standards (items 13.a, b or c) in the 600-800K range, test in triplicate and determine average _____

2. Optionally, prepare sufficient control/sample 600-800K range, test in triplicate and determine average _____

START UP

14. Daily Instrument Start-up _____

a. Check that the volume of rinse/sheath liquid in the supply containers is sufficient for the number of samples to be tested. Rinse/sheath liquid must be completely replaced, leftover discarded, every 7 days or when volume too low, see item 11.a _____

b. Solutions not used beyond expiration date(s) _____

c. Activate measure mode on instrument _____

d. Prepare Bacterial Control Sample (BCS) (item 10.b.1 or 10.b.2) as the instrument goes into measure mode _____

e. Perform quality check _____

1. Blank check: Test 2 blank solutions (item 10.a) measured in SCC and IBC mode, respectively. The mean SCC count must be ≤3,000 cells/mL and individual measurements <5,000 cells/mL, all bacteria results must be ≤2 CFU

2. **IF ANY ABOVE PARAMETERS ARE OUT OF VARIANCE, CORRECT BEFORE PROCEEDING** _____

3. BCS samples _____

a. Run BCS with 2 intakes _____

b. Check that the results of the Bacterial Control Sample (item 10.b.1. or item 10.b.2) conform to the specified limits. The Laboratory Average Count must be within the Manufacturer Count Limits and the Laboratory Average Signal Mean must be within the Manufacturer Provided Average Signal Mean (±2) _____

Manufacturer Provided Average Count	_____	_____
Manufacturer Provided Count Limits	_____	_____
Laboratory Average Count	_____	_____
Manufacturer Provided Average Signal Mean	_____	_____
Laboratory Average Signal Mean	_____	_____

c. If the BCS sample is outside the specified limits, and does not correct after re-measurement, follow instructions in User Manual or seek technical assistance _____

4. SCC standard _____

a. Mix by inverting at least 25 times _____

b. Test all of the 4 standards. Test 1 of them in triplicate for repeatability; maintain records _____

c. The standard's average must be within 10% of the DMSCC (item 13) for that level, except within 15% for 100K-200K standard; maintain records _____

d. Repeatability - a standard in the 300K to 800K range must have a coefficient of variation (CV) of 5% or less on 10 replicates **(Refer to Operating Manual)**; maintain records _____

e. Maintain records on all parameters each time instrument is used _____

f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

15. Handling Samples _____

a. If bacteria testing is required, samples must first be tested for the presence of inhibitors prior to analysis on the BacSomatic _____

b. Samples kept at 0.0-4.5°C until tested _____

16. Testing Samples _____

a. Before placing the sample under the instrument's pipette, invert it no less than 25 times to mix properly _____

b. Start testing procedure immediately _____

c. Record number of somatic cells and bacteria counted for each sample _____

17. Records _____

a. Maintain records of all results, controls and samples _____

18. Follow End of Day Shut-Down and Cleaning _____

a. Perform quality check _____

1. Blank check: Test 2 blank solutions (item 10.a) measured in SCC and IBC mode, respectively. The mean SCC count must be ≤3,000 cells/mL and individual measurements <5,000 cells/mL, all results must be ≤2 CFU _____

2. BCS sample _____

a. Run BCS with 2 intakes _____

b. Check that the results of the Bacterial Control Sample (item 10.b.1. or item 10.b.2) conform to the specified limits. The Laboratory Average Count must be within the Manufacturer Count Limits and the Laboratory Average Signal Mean must be within the Manufacturer Provided Average Signal Mean (±2) _____

Manufacturer Provided Average Count _____ _____

Manufacturer Provided Count Limits _____ _____

Laboratory Average Count _____ _____

Manufacturer Provided Average Signal Mean _____ _____

Laboratory Average Signal Mean _____ _____

c. If the BCS sample is outside the specified limits, and does not correct after re-measurement, follow instructions in User Manual or seek technical assistance _____

3. SCC standard _____

a. Mix by inverting at least 25 times _____

b. Test 1 standard in duplicate, average the counts for this level; maintain records _____

b. Place the sample vial containing End of Day solution (item 10.c) under the pipette _____

c. Activate/go to stop mode on instrument _____

d. Remove waste funnel and clean sample collection funnel as described in manufacturer's instrument User Manual _____

19. Routine Maintenance _____

a. Maintain records _____

REPORTING

20. Computing and Reporting Counts _____

a. Somatic Cell Count _____

1. Count obtained x 1000 is the cell count/mL milk _____

2. In reporting electronic somatic cell counts (ESCC/mL); record only first two left hand digits, raising second digit to next higher number when third digit is 6 or more _____

3. Report the two left hand digits (rounded) _____

a. If the third digit is 5 the second digit is rounded by the following rule _____

1. When the second digit is odd round up, raise the second digit by 1 (odd up, 235 to 240) _____

2. When the second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220) _____

4. If count on instrument is < 100 report as < 100,000 ESCC/mL _____

b. Bacterial counting _____
[When samples are demonstrated to contain inhibitors, no bacteria counts are reported; report as positive for inhibitors or growth inhibitors (GI)]

1. Unit

   a. The readout is in IBC (Individual Bacteria Counts)/µL _____

   b. IBC is converted using the conversion table entered into the instrument and is reported in the result list as CFU/µL _____

   c. Proper conversion factor has been entered for the regulatory range _____

2. Reporting

   a. Report the bacterial content of the milk as BacSomatic CFU/mL (CFU/µL x 1000 = CFU/mL) _____

   b. Instrument reports in CFU/µL, laboratory analyst must convert to CFU/mL for official reporting _____

3. Report the two left hand digits (rounded) _____

   a. If the third digit is 5 the second digit is rounded by the following rule _____

      1. When the second digit is odd round up, raise the second digit by 1 (odd up, 235 to 240) _____

      2. When the second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220) _____