

**FOSSOMATIC BACSOMATIC™**

**(Raw Commingled Cow Milk)  
IMS # 7e & 16h**

**(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<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) \_\_\_\_\_
  - 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 ( $\pm$  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 ( $\pm$  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  $\leq 3,000$  cells/mL and individual measurements  $< 5,000$  cells/mL, all bacteria results must be  $\leq 2$  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 ( $\pm 2$ ) \_\_\_\_\_
 

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  $\leq 3,000$  cells/mL and individual measurements  $< 5,000$  cells/mL, all results must be  $\leq 2$  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 ( $\pm 2$ ) \_\_\_\_\_

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)/ $\mu$ L \_\_\_\_\_

b. IBC is converted using the conversion table entered into the instrument and is reported in the result list as CFU/ $\mu$ L \_\_\_\_\_

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/ $\mu$ L x 1000 = CFU/mL) \_\_\_\_\_

b. Instrument reports in CFU/ $\mu$ L, 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) \_\_\_\_\_