

ELECTRONIC SOMATIC CELL COUNT

Fossomatic™ 5000/FC (Raw Commingled Cow, Sheep, Goat, Water Buffalo and Camel Milk) IMS #16

(Unless otherwise stated all tolerances $\pm 5\%$)

1. **Laboratory Requirements (see Cultural Procedures (CP) items 33 & 34)** _____
 - a. Un-preserved samples may be run up to 72 hours after initial collection _____
 - b. 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 ($K_2Cr_2O_7$) _____

2. **Comparative Test with DMSCC**
[NOT required as a co-requisite for certification of analysts in laboratories purchasing standards from a CERTIFIED provider (item 12.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 _____
 - c. Required for laboratories preparing in house standards or using commercially prepared standards (items 12.a and c) and for those testing goat or camel milk _____

APPARATUS

3. **See CP items 1-4** _____
4. **Electronic Somatic Cell Counter** _____
 - a. Fossomatic FC _____
 - b. Fossomatic 5000 _____
5. **Water Bath** _____
 - a. Circulating and thermostatically controlled to 37-42°C _____

REAGENTS

6. Reagents

- a. Buffer 5000, Reagent E Lot #: _____ Exp. Date: _____
- b. Clean 5000, Reagent D Lot #: _____ Exp. Date: _____
- c. Dye 5000, Reagent B Lot #: _____ Exp. Date: _____

7. Other Solutions

- a. Blank solution: Prepare a 1% (w/v) NaCl solution, MilkoScan Rinse Solution or 0.5% S-470 solution

8. Preparation of Reagents for the Fossomatic FC

- a. 1 L bags

- 1. Stock Solution: Dissolve 500 mL of Clean 5000, Reagent D, in 4.5 L of deionized (DI) or MS water, heat to about 60°C, store in airtight, light proof container in a cool location and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- 2. Buffer/diluent Solution: Mix 1 L of stock solution (item 8.a.1) with one bag (354 g) of Buffer 5000, Reagent E, add DI or MS water to 10 L, heat to 40-60°C to speed process, store in buffer/diluent container next to instrument and use within 6 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- 3. Rinse/sheath Liquid: Mix 250 mL of stock solution (item 8.a.1) with DI or MS water to make 50 L, store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- 4. Insert Dye 5000 bag, Reagent B, according to manufacturer's instructions

- b. 0.5 L Bags

- 1. Stock Solution: Dissolve 100 mL of Clean 5000, Reagent D, in 900mL of DI or MS water, heat to about 60°C, store in airtight, light proof container in a cool location and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

2. Buffer/diluent Solution: Mix 0.5 L of stock solution (item 8.b.1) with one bag (171 g) of Buffer 5000, Reagent E, add DI or MS water to 5 L, heat to 40-60°C to speed process, store in buffer/diluent container next to instrument and use within 6 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

3. Rinse/sheath Liquid: Mix 100 mL of stock solution (item 8.b.1) with DI or MS water to 20 L store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

4. Insert Dye 5000 bag, Reagent B, according to manufacturer's instructions

9. Preparation of Reagents for the Fossomatic 5000

a. Stock Solutions for the Fossomatic 5000

1. Dye stock solution: Dissolve 3 ethidium bromide tablets in 1 L of DI or MS water, stir to completely dissolve tablets, store in dark cool location and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

2. Clean 5000 stock solution: Dilute one bottle of Clean 5000, Reagent D, in 4.5 L of DI or MS water, heat to about 40-60°C to speed process, store and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

b. Working Solutions for the Fossomatic 5000

1. Buffer/diluent solution: Dissolve one bag of Buffer 5000, Reagent E, in approximately 8 L of DI or MS water in 10 L container, add 1 L of Clean 5000 stock solution (item 9.a.2) and fill to a total of 10 L with DI or MS water, store and use within 6 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

2. Dye/buffer solution: Mix 1800 mL of Buffer/diluent solution (item 9.b.1) and 200 mL of Dye stock solution (item 9.a.1) in the reagent bottle and place in the instruments according to instructions and use within 6 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

3. Rinse/sheath liquid: Fill a 50 L container with approximately 49 L of DI or MS water, add 250 mL of Clean 5000 stock solution (item 7.a.2) and fill to 50 L with DI water and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

10. All solutions labeled with date prepared and expiration date _____

START UP

11. Cell Counter _____

- a. Check that the volume of rinse/sheath liquid, dye and buffer solutions in the supply containers is sufficient for the number of samples to be tested _____
- b. Solutions not used beyond expiration date(s) _____
- c. Turn power on and place instrument in standby mode _____
- d. Perform a blank check: Test the blank solution (item 7.a). The mean count must be $\leq 3,000$ cells/mL and individual measurements $< 5,000$ cells/mL _____
- e. **IF ANY ABOVE PARAMETERS ARE OUT OF VARIANCE, CORRECT BEFORE PROCEEDING** _____
- f. Maintain records on all parameters each time instrument is used _____

12. 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 12.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 _____

PROCEDURE

13. Testing Standards (each time instrument used) _____

- a. Heat standards to 37-42°C (using a temperature control) and test within 30 min of reaching temperature, use once and then discard, i.e. do not re-use _____
- b. Mix by inverting at least 2x, test standards within 3 min _____
- c. Test the standards in triplicate and average the counts for each level; maintain records _____

- d. Each standard's average must be within 10% of the DMSCC (item 12) for that level, except within 15% for 100K-200K standard; maintain records _____
- e. 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 _____
- f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

14. Testing Samples _____

- a. Heat samples to 37-42°C (using a temperature control) and test within 30 min of reaching temperature _____
- b. Test samples within 10 min after removal from water bath _____
- c. Mix by inverting at least 2x, test samples within 3 min _____
- d. Record number of cells counted for each sample _____

15. With Continuous Operation: _____

- a. Perform a blank check (item 11.d) hourly _____
- b. Test a standard or optionally a control/sample (item 12.d) in the 600K to 800K range hourly in triplicate and determine the average, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average) _____
- c. Maintain records _____

16. Routine Maintenance _____

- a. Maintain records _____

REPORTING

17. Computing and Reporting Counts _____

- a. Count obtained x 1000 is the cell count/mL milk _____
- b. 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 _____
- c. Report the two left hand digits (rounded) _____
 - 1. If the third digit is 5 the second digit is rounded by the following rule _____
 - a. When the second digit is odd round up, raise the second digit by 1 (odd up, 235 to 240) _____

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

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

e. If goat or camel milk is over the regulatory limit, follow confirmation procedure in PMO
