

ELECTRONIC SOMATIC CELL COUNT

Fossomatic™ 5000/FC/7 (Raw Commingled Cow, Sheep, Goat, Water Buffalo and Camel Milk) IMS #16d

(Unless otherwise stated all tolerances are ±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 (K₂Cr₂O₇) _____

- 2. **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 _____
 - c. Required for laboratories preparing in house standards or using commercially prepared standards (items 13.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-7 _____
 - b. Fossomatic FC _____
 - c. Fossomatic 5000 _____

5. Water Bath

- a. Circulating and thermostatically controlled to 37-42°C

REAGENTS

6. Reagents

- a. Fossomatic Buffer/Buffer 5000, Reagent E

Lot #: _____ Exp. Date: _____

- b. Fossomatic Buffer Small (alternately)

Lot #: _____ Exp. Date: _____

- c. Fossomatic Detergent/Clean 5000, Reagent D

Lot #: _____ Exp. Date: _____

- d. Fossomatic Dye/Dye 5000, Reagent B

Lot #: _____ Exp. Date: _____

- e. Fossomatic Dye Concentrate (for Fossomatic 7 with manual mixing option)

Lot #: _____ Exp. Date: _____

7. Other Solutions

- a. Fossomatic 7

1. Blank Solution: Prepare Rinse/sheath liquid (item 8.a.1.c or 8.a.2.c)

- b. Fossomatic FC and 5000

1. 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 7

a. Automatic reagent mixing module

[There are two ways (i.e. high and low capacity) to prepare reagents according to the capacity requirements of the respective laboratory]

1. High capacity:

- a. Stock Solution: Heat 500 mL of Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 500 mL Fossomatic Detergent with 4.5 L of deionized (DI) or MS water; store in airtight, lightproof container in a cool location and use within 16 weeks.

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Buffer/Diluent Solution: Mix 1 L of Stock Solution (item 8.a.1.a) with one bag (354 g) of Fossomatic Buffer (item 6.a) add DI or MS water to 10 L, heat to 50 - 60°C to speed process; store in buffer/diluent container next to instrument and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- c. Rinse/Sheath Liquid: Mix 250 mL of Stock Solution (item 8.a.1.a) with DI or MS water to make 50 L; store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- d. Insert Fossomatic Dye bag according to manufacturer's instructions

2. Low-capacity

- a. Stock Solution: Heat Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 125 mL of Fossomatic Detergent with 1125 mL of DI or MS water; store in airtight, light proof container in a cool location and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Buffer/Diluent Solution: Mix 200 mL of Stock Solution (item 8.a.2.a) with one bag (70.8 g) of Fossomatic Buffer Small (item 6.b) add DI or MS water to 2 L, heat to 40-60°C to speed process; store buffer/diluent container next to instrument and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- c. Rinse/Sheath Liquid: Mix 100 mL of Stock Solution (item 8.a.2.a) with DI or MS water to make 20 L; store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- d. Fossomatic Dye Working Solution: Mix one bottle (200 mL) of Fossomatic Dye Concentrate (item 6.e) with 400 mL of Buffer/Diluent Solution (item 8.b.2) and fill up to the 2 L mark with Buffer/Diluent Solution

Lab Prep Date: _____ Lab Exp. Date: _____

b. Manual reagent mixing module

- 1. Stock Solution: Heat Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 minutes. Mix 125 mL of Fossomatic Detergent with 1125 mL of DI or MS water; 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 200 mL of Stock Solution (item 8.b.1) with one bag (70.8 g) of Fossomatic Buffer Small (item 6.b), add DI or MS water to 2 L, heat to 40-60°C to speed process; store buffer/diluent container next to instrument and use within 3 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 make 20 L; store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- 4. Fossomatic Dye Working Solution: Mix one bottle (200 mL) of Fossomatic Dye Concentrate (item 6.e) with 400 mL of Buffer/Diluent Solution (item 8.b.2) and fill up to the 2 L mark with Buffer/Diluent Solution

Lab Prep Date: _____ Lab Exp. Date: _____

9. Preparation of Reagents for the Fossomatic FC

a. High Capacity

1. Stock Solution

- a. Stock Solution using Fossomatic Detergent: Heat 500 mL of Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 500 mL Fossomatic Detergent with 4.5 L of DI or MS water; store in airtight, light proof container in a cool location and use within 16 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Stock Solution using Clean 5000, Reagent D: Dissolve 500 mL of Clean 5000, Reagent D (item 6.c) in 4.5 L of DI or MS water, heat to about 60°C; store in airtight, lightproof 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 9.a.1.a. or 9.a.1.b) 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 9.a.1.a. or 9.a.1.b) 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, Reagent B bag (item 6.d) according to manufacturer's instructions

b. Low Capacity

1. Stock Solution

- a. Stock Solution using Fossomatic Detergent: Heat Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 125 mL of Fossomatic Detergent with 1125 mL of DI or MS water; store in airtight light proof container in a cool location and use within 16 weeks.

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Stock Solution using Clean 5000/Reagent D: Dissolve 100 mL of Clean 5000, Reagent D (item 6.c) in 900 mL 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 9.b.1.a or 9.b.1.b) 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 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

3. Rinse/Sheath Liquid: Mix 100 mL of Stock Solution (item 9.b.1.a or 9.b.1.b) with DI or MS water to 20 L; store and use within 3 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

4. Insert Dye 5000, Reagent B bag (item 6.d) according to manufacturer's instructions _____

10. Preparation of Reagents for the Fossomatic 5000 _____

- a. Automatic reagent mixing module
[There are two ways (i.e. high and low capacity) to prepare reagents according to the capacity requirements of the respective laboratory] _____

1. High capacity: _____

- a. Stock Solution _____

1. Stock Solution using Fossomatic Detergent: Heat 500 mL of Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 500 mL Fossomatic Detergent with 4.5 L of DI or MS water; store in airtight, lightproof container in a cool location and use within 16 weeks. _____

Lab Prep Date: _____ Lab Exp. Date: _____

2. Stock Solution using Clean 5000, Reagent D: Dissolve 500 mL of Clean 5000, Reagent D (item 6.c) in 4.5 L of DI or MS water, heat to about 60°C; store in airtight, lightproof container in a cool location and use within 16 weeks. _____

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Buffer/Diluent Solution: Mix 1 L of Stock Solution (item 10.a.1.a.1 or 10.a.1.a.2) with one bag (354 g) of Fossomatic Buffer (item 6.a) add DI or MS water to 10 L, heat to 50 - 60°C to speed process; store in buffer/diluent container next to instrument and use within 3 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

- c. Rinse/Sheath Liquid: Mix 250 mL of Stock Solution (item 10.a.1.a.1 or 10.a.1.a.2) with DI or MS water to make 50 L; store and use within 3 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

- d. Insert Fossomatic Dye bag according to manufacturer's instructions _____

2. Low-capacity _____

a. Stock Solution _____

1. Stock Solution using Fossomatic Detergent: Heat Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 125 mL of Fossomatic Detergent with 1125 mL of DI or MS water; store in airtight light proof container in a cool location and use within 16 weeks. _____

Lab Prep Date: _____ Lab Exp. Date: _____

2. Stock Solution using Clean 5000/Reagent D: Dissolve 100 mL of Clean 5000, Reagent D (item 6.c) in 900 mL 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: _____

- b. Buffer/Diluent Solution: Mix 200 mL of Stock Solution (item 10.a.2.a.1 or 10.a.2.a.2) with one bag (70.8 g) of Fossomatic buffer Small (item 6.b) add DI or MS water to 2 L, heat to 40-60°C to speed process; store buffer/diluent container next to instrument and use within 3 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

- c. Rinse/Sheath Liquid: Mix 100 mL of Stock Solution (item 10.a.2.a.1 or 10.a.2.a.2) with DI or MS water to make 20 L; store and use within 3 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

- d. Fossomatic Dye Working Solution: Mix one bottle (200 mL) of Fossomatic Dye Concentrate (item 6.e) with 400 mL of Buffer/Diluent Solution (item 10.a.2.b) and fill up to the 2 L mark with Buffer/Diluent Solution _____

Lab Prep Date: _____ Lab Exp. Date: _____

b. Manual reagent mixing module _____

1. Stock Solution: _____

- a. Stock Solution using Fossomatic Detergent: Heat Fossomatic Detergent (item 6.c) in 40°C water bath until solution's appearance is clear, time not to exceed 10 min. Mix 125 mL of Fossomatic Detergent with 1125 mL of DI or MS water; store in airtight lightproof container in a cool location and use within 16 weeks _____

Lab Prep Date: _____ Lab Exp. Date: _____

- b. Stock Solution using Clean 5000/Reagent D: Dissolve 100 mL of Clean 5000, Reagent D (item 6.c) in 900 mL 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 200 mL of Stock Solution (item 10.b.1.a or 10.b.1.b) with one bag (70.8 g) of Fossomatic Buffer Small (item 6.b) add DI or MS water to 2 L, heat to 40-60°C to speed process; store buffer/diluent container next to instrument and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

3. Rinse/sheath Liquid: Mix 100 mL of Stock Solution (item 10.b.1.a or 10.b.1.b) with DI or MS water to make 20 L; store and use within 3 weeks

Lab Prep Date: _____ Lab Exp. Date: _____

4. Fossomatic Dye Working Solution: Mix one bottle (200 mL) of Fossomatic Dye Concentrate (item 6.e) with 400 mL of Buffer/Diluent Solution (item 10.b.2) and fill up to the 2 L mark with Buffer/Diluent Solution

Lab Prep Date: _____ Lab Exp. Date: _____

11. All solutions labeled with date prepared and expiration date

START UP

12. 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 or 7.b). 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

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

PROCEDURE

14. 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 13) 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**

15. 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

16. With Continuous Operation:

- a. Perform a blank check (item 12.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

17. Routine Maintenance

- a. Maintain records

REPORTING

18. 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 the PMO