

# 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

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