ELECTRONIC SOMATIC CELL COUNT

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

(Unless otherwise stated all tolerances ±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 ($K_2Cr_2O_7$) _____

PRE-REQUISITE

2. Comparative Test with DMSCC (co-requisite for certification) _____

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 State/Federal 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

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 Liter 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 Liter 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 deionized 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 ≤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 _____

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 <u>cannot</u> 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 (C_V) 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 _____

REPORTS

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 milk is over the regulatory limit, follow confirmation procedure in PMO _____