

ELECTRONIC SOMATIC CELL COUNT

Fossomatic™ 250/300/360/400 (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 tested 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 15.b)] _____
 - a. Analysts 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 file in laboratory); kept for as long as analyst is certified _____
 - c. Required for laboratories preparing in house standards or using commercially prepared standards (items 15.a and c) and for those testing goat or camel milk _____

APPARATUS

3. **See CP items 1-4** _____

4. **Automated Electronic Somatic Cell Counters** _____
 - a. Fossomatic 250 _____
 - b. Fossomatic 300 _____
 - c. Fossomatic 360 _____
 - d. Fossomatic 400 _____

5. Water Bath

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

REAGENTS

6. Stock Dye/Buffer Solution (caution TOXIC, use gloves when handling and do not breathe dust)

- a. Dissolve 2.5 g (or number of tablets specified by manufacturer) ethidium bromide ($C_{21}H_{20}BrN_3$) in 1 L deionized (DI) or MS water by heating to 40-60°C and mix to dissolve
- b. Add 400 g tripotassium citrate monohydrate ($C_6H_5O_7K_3 \cdot H_2O$), 14.5 g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$), and 4 L DI or MS water, heat to 40-60°C and mix to dissolve
- c. Add dye and buffer solutions together and mix
- d. Add 50 mL neutral detergent, Triton X-100 to mixture and stir until dissolved
- e. Store refrigerated (0.0-4.5°C) in airtight, light-proof container for no longer than 90 days

Lab Prep. Date: _____ Exp. Date: _____

7. Stock Detergent Solution

- a. Dissolve 10 mL neutral detergent, Triton X-100 in 1 L of DI or MS water and heat 40-60°C to complete solution
- b. Store refrigerated (0.0-4.5°C) in airtight, container for no longer than 30 days

Lab Prep. Date: _____ Exp. Date: _____

8. Ammonium Hydroxide (NH₄OH) Solution, Reagent Grade, 25%

9. All stock dye/buffer and detergent solutions labeled with date prepared and expiration date

WORKING SOLUTIONS

10. Dye/Buffer Solution

- a. Dilute 1 L dye/buffer stock solution (item 6) with 9 L DI or MS water
- b. Protect from light and use within 21 days

Lab Prep. Date: _____ Exp. Date: _____

11. Rinsing Solution (use within 7 days)

- a. Add 10 mL of stock neutral detergent stock solution (item 7) and 25 mL of ammonium hydroxide solution (item 8) and suspend to 10 L with DI or MS water

Lab Prep. Date: _____ Exp. Date: _____

12. Optionally, use manufacturer's reagent kits and instructions specific for each Instrument

13. All working dye/buffer and rinsing solutions labeled with date prepared and expiration date

START UP

14. Cell Counter

- a. Check that the amount of dye/buffer solution (item 10) and rinsing (cleaning) solution (item 11) in instrument supply containers is of sufficient volume for the number of samples to be tested
- b. Solutions not used beyond expiration date(s)
- c. Turn on power and cycle at least six times
- d. Perform a zero check before starting any measurements, within acceptable limits, single counts up to 5 and mean up to 3
- e. **IF ANY ABOVE PARAMETERS ARE OUT OF VARIANCE, CORRECT BEFORE PROCEEDING**
- f. Maintain records on all parameters each time instrument is used

15. 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, preserve 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, used within one week _____

Lab Prep. Date: _____ 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 15.a, b or c) in the 600-800K range, run in triplicate and determine average _____
2. Optionally, prepare sufficient control/sample 600-800K range, run in triplicate and determine average _____

PROCEDURE

16. 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, used once and then discarded; i.e. do not re-use
- b. Mix by inverting at least 2x, place in rack and put onto automatic track, run within 10 min
- c. Run the standards six times and average the counts for each level; maintain records
- d. Each standard's average must be within 10% of the DMSCC (item 15) 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**

17. Testing Samples

- a. Heat samples to 37-42°C (using a temperature control) and read within 30 min of reaching temperature
- b. Samples must not be re-used and must be discarded after use
- c. Mix by inverting at least 2x, place in rack and put onto automatic track, run within 10 min of reaching the testing temperature

18. With continuous operation:

- a. Run a standard or optionally a control/sample (item 15.d) in the 600K to 800K range hourly, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average)
- b. Run control 6x
- c. Run zero control (item 14.d)
- d. Maintain records

19. Routine maintenance

- a. Maintain records

REPORTING

20. 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