ELECTRONIC SOMATIC CELL COUNT

Fossomatic™ 250/300/360/400
(Raw Commingled Cow, Sheep, Goat, Water Buffalo and Camel Milk)
IMS #16b

(Unless otherwise stated all tolerances ±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 ($C_6H_5O_7K_3 \bullet H_2O$), 14.5 g citric acid monohydrate ($C_6H_8O_7 \bullet H_2O$), 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 (NH_4OH) 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 _____