ELECTRONIC SOMATIC CELL COUNT

Fossomatic™ 90
(Raw Commingled Cow, Sheep, Goat, Water Buffalo and Camel 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 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 17.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 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 17.a and c) and for those testing goat or camel milk _____

APPARATUS

3. See CP items 1-4 _____

4. Fossomatic 90 Electronic Somatic Cell Counter _____

5. Water Bath _____

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

6. Pipettor, fixed volume or electronic (_____) _____

a. Calibrate to deliver 500 µL milk (see CP item 6.e) _____

b. Maintain records _____

7. Pipettor Tips _____

a. Disposable, replace for each sample _____

REAGENTS

8. Stock Dye Solution, 0.1% Ethidium Bromide (caution TOXIC, use gloves when handling and do not breathe dust) _____

a. Dissolve 1.0 g ethidium bromide ($C_{21}H_{20}BrN_3$) in 1 L DI or MS water by heating to 40-60°C _____

b. Store in light-proof, air-tight bottle no more than 60 days _____

c. Lab Prep. Date: _____ Exp. Date: _____ _____

9. Stock Rinsing Solution, 1% Triton X-100 _____

a. Dissolve 10 mL Triton X-100 in 1 L DI or MS water by heating to 60°C _____

b. Store in air-tight container no more than 25 days _____

c. Lab Prep. Date: _____ Exp. Date: _____ _____

10. Stock Buffer Solution, 0.025 M Potassium Hydrogen Phthalate _____

a. Dissolve 51.0 g KH phthalate and 13.75 g KOH in 10 L DI or MS water by heating to 40-60°C _____

b. Add 150 mL 1% Triton X-100 (item 9), store less than 7 days in airtight container _____

c. Lab Prep. Date: _____ Exp. Date: _____ _____

11. Ammonium Hydroxide (NH_4OH) Solution, Reagent Grade, 25% _____

WORKING SOLUTIONS

12. Working Dye Solution/Zero Control (used within 7 days) _____

a. Dilute 26 mL stock dye solution (item 8.a) to 2.5 L with stock buffer solution (item 10.b) _____

b. Lab Prep. Date: _____ Exp. Date: _____ _____

13. Working Rinsing Solution (used within 7 days) _____

a. Add 10 mL stock rinsing solution (item 9) to 25 mL of 25% NH_4OH and dilute to 10 L with DI or MS water _____

b. Lab Prep. Date: _____ Exp. Date: _____ _____

14. All solutions labeled with date prepared and expiration date _____

15. Optionally use manufacturer's reagent kits and instructions specific for each Instrument _____

START UP

16. Cell Counter _____

a. Assure adequate volume of working solutions, not used beyond expiration date(s) _____

b. Turn on power and cycle at least six times _____

c. Blind count ≤ 5 _____

d. Vacuum pressure setting minimum of -40 kPa _____

e. Dispenser filling time 4-5 sec _____

f. Intake filling time 3-4 sec _____

g. **IF ANY ABOVE PARAMETERS ARE OUT OF VARIANCE, CORRECT BEFORE PROCEEDING** _____

h. Maintain records on all parameters _____

17. 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. Use standards 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. Use standards 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-brono-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: _____ Exp. Date: _____ _____
5. Perform DMSCC in triplicate on each standard prepared 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 17.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

18. 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. Invert 10 times, pipet 500 µL into intake chamber within 3 min _____

c. Run 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 17) for that level, except within 15% for 100-200K 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** _____

19. Testing Samples _____

a. Heat samples to 37-42°C (using a temperature control) and test within 30 min of reaching temperature _____

b. Samples must not be re-used and must be discarded after use _____

c. Invert 10 times, pipet 500 µL into intake chamber within 3 min _____

d. Record number of cells counted for each sample _____

20. With Continuous Operation: _____

a. Perform a zero check (item 17.d)

b. Test a standard or optionally a control/sample (item 17.d) in the 600 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 _____

21. Routine maintenance _____

a. Maintain records _____

REPORTING

22. 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 _____