

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 (K₂Cr₂O₇) _____

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 (NH₄OH) 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% NH₄OH 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-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: _____ 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