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 ±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 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 ($C_6H_5O_7K_3\cdotH_2O$), 14.5 g citric acid monohydrate ($C_6H_8O_7\cdotH_2O$), 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/river 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

   Solutions not used beyond expiration date(s)

   Turn on power and cycle at least six times

   Perform a zero check before starting any measurements, within acceptable limits, single counts up to 5 and mean up to 3

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

   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$_2$Cr$_2$O$_7$)

        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