

ELECTRONIC SOMATIC CELL COUNT

**Bentley Somacount™ 150/300/500/FCM
(Raw Cow Milk, Raw Sheep Milk, Raw Goat Milk and Raw Water Buffalo 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 run 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) _____

PRE-REQUISITE

- 2. **Comparative Test with DMSCC (co-requisite for certification)** _____
- a. Analyst(s) 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 on file in the laboratory); kept for as long as analyst is certified _____

APPARATUS

- 3. **See CP Items 1-4** _____
- 4. **Electronic Somatic Cell Counter** _____
 - a. Bentley Somacount 150 _____
 - b. Bentley Somacount 300 _____
 - c. Bentley Somacount 500 _____
Dual Channel Machine (DCM) _____
 - d. Bentley Somacount FCM _____
Dual Channel Machine (DCM) _____

5. Water Bath

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

REAGENTS

6. Stock Dye/Buffer Solution

- a. Dissolve 80g of tripotassium citrate monohydrate, ($K_3C_6H_5O_7 \cdot H_2O$), 3.0g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$), and 0.25g (1 tablet) of ethidium bromide ($C_{21}H_{20}BrN_3$) in 750 mL of deionized (DI) or MS water. Heat to 40-60°C and stir until totally dissolved
- b. Add 10 mL of neutral detergent, Triton X-100, and stir until totally dissolved. Adjust volume to 1 Liter with DI or MS water
- c. Store refrigerated (0-4.5°C) in airtight, light-proof container for no longer than 90 days

Lab Prep Date: _____ Lab Exp. Date: _____

WORKING SOLUTIONS

7. Dye/Buffer Solution

- a. Dilute 1 part of Stock Dye/Buffer solution with 9 parts of DI or MS water
- b. Protect from light and use within 21 days

Lab Prep Date: _____ Lab Exp. Date: _____

8. Rinse Solution

- a. Add 20 mL of alkaline detergent, RBS-35, per liter of DI or MS water and mix
- b. Use within 7 days

Lab Prep Date: _____ Lab Exp. Date: _____

9. Optionally, Use Manufacturer's Reagent Kits and Instructions

10. All Solutions Labeled with Date Prepared and Expiration Date

START UP

11. Cell Counter

- a. Check that the volume of dye/buffer solution (item 7) and rinse solution (item 8) in the supply containers is of sufficient volume for the number of samples to be tested
- b. Solutions not to be used beyond expiration date(s)

- c. Turn on computer and instrument, wait 20 minutes before proceeding _____
- d. Laser power > 0.25 mW _____
- e. |PMT voltage| > 10 mV _____
- f. Dye/Coil temperature between 67-73°C _____
- g. Test DI or MS water at least 3 times on each channel in use; (i.e. 6 times for dual channel instruments) reading must be zero (0) on every test _____
- h. **IF ANY ABOVE PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** _____
- i. Maintain records on all parameters each time instrument is used _____

12. 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 _____

- c. Laboratory prepared (weekly) _____
 - 1. Prepare from raw milk >18 hours old preserved with 0.05% potassium dichromate ($K_2Cr_2O_7$) _____
 - 2. Or, preserved 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: _____ Lab 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 12.a, b or c) in the 600-800K range; test in triplicate and determine average _____
 - 2. Optionally, prepare sufficient control/sample 600-800K range, test in triplicate and determine average _____

PROCEDURE

13. 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 discard; i.e., do not re-use _____
- b. Mix by inverting at least 2x, test standards within 3 min _____
- c. Test the 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 12) 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 (C_v) of 5% or less on 10 replicates (**Refer to Operating Manual**); maintain records _____
- f. For dual channel machines, the standards must be run in triplicate on each channel and coefficient of variation (C_v) must be determined for each channel that is in use _____
- g. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

- h. Dual Channel Machines (DCM) can be run on single channel _____
 - 1. Switch off channel that does not meet above parameters per operating instructions _____
 - 2. Run machine on single channel _____

14. Testing Samples _____

- a. Heat samples to 37-42°C (using a temperature control) and read within 30 min of reaching temperature _____
- b. Test samples within 10 min after removal from water bath _____
- c. Mix by inverting at least 2x, test samples within 3 min _____
- d. Record number of cells counted for each sample _____

15. With Continuous Operation: _____

- a. Run zero control (item 11.g) hourly _____
- b. Test a standard or optionally a control/sample (item 11.d) in the 600K 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. For dual channel machines, the hourly control in triplicate and the zero control must be tested and found acceptable for each channel that is in use _____
- e. Maintain records _____

16. Routine Maintenance _____

- a. Maintain records _____

REPORTS

17. Computing and Reporting of 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) _____
 - 2. If the third digit is 5, the second digit is rounded by the following rule _____
 - a. When second digit is odd round up, raising the second digit by 1 (odd up, 235 to 240) _____

b. When 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 count as < 100,000 ESCC/mL _____

e. If goat milk is over the regulatory limit, follow confirmation procedure in PMO _____