ELECTRONIC SOMATIC CELL COUNT

Bentley Somacount™ 150/300/500/FCM
(Raw Commingled Cow, Goat, Sheep, 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 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 ($K_2Cr_2O_7$) _____

2. Comparative Test with DMSCC
[NOT required as a co-requisite for certification of analysts in laboratories purchasing standards from a CERTIFIED provider (item 12.b)] _____

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 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 12.a and c) and for those testing goat or camel milk _____

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 80 g of tripotassium citrate monohydrate, ($K_3C_6H_5O_7,H_2O$), 3.0 g of citric acid monohydrate ($C_6H_8O_7,H_2O$), and 0.25 g (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 _____

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, preserved 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: _____ 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 (CV) 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 (CV) 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 _____

REPORTING

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) _____

1. 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 or camel milk is over the regulatory limit, follow confirmation procedure in PMO _____