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 (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 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, (K₃C₆H₅O₇·H₂O), 3.0 g of citric acid monohydrate (C₆H₈O₇·H₂O), and 0.25 g (1 tablet) of ethidium bromide (C₂₁H₂₀BrN₃) 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 (K₂Cr₂O₇)
      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 (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