

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION <b>MILK LABORATORY EVALUATION FORM</b>	LABORATORY	
	LOCATION	LAB #
	DATE	X = DEVIATION      U = UNDETERMINED O = NOT USED      NA = NOT APPLICABLE

**ELECTRONIC SOMATIC CELL COUNT**  
**Bentley Somacount 150/300/500**  
 (Unless other wise stated all tolerances ±5%)

1. **Laboratory requirements (see CP, item 33 and 34)** .....
  - a. Unpreserved samples may be run up to 72 hours after initial collection
  - b. Samples may be run 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<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) .....
  - c. Comparative test with DMSCC .....
    1. Performed by each analyst performing ESCC test .....
    2. Test 4 samples (100K - 200K, 300K - 500K, 600K - 800K and 900K - 1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC (three separate subsamples each, do not read same sample three times) .....
    3. Results must be shown to be acceptable prior to official testing by analyst performing comparison, i.e. analyst is not certified until found acceptable. **(co-requisite for certification)** .....
    4. Copy of comparison and results in QC record (or easily accessible file in laboratory) .....
  - d. Analysts certified in DMSCC .....

**APPARATUS**

2. **Cultural procedures, items 1 - 5** .....
3. **Electronic Somatic Cell Counter** .....
  - a. Bentley Somacount™ 150 .....
  - b. Bentley Somacount™ 300 .....
  - c. Bentley Somacount™ 500 .....
4. **Water bath** .....
  - a. Circulating and thermostatically controlled to 37 - 42C .....

**REAGENTS**

5. **Stock Dye/Buffer Solution** .....
  - a. Dissolve 80g of tripotassium citrate monohydrate, (K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·H<sub>2</sub>O), 3.0g of citric acid monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·H<sub>2</sub>O), and 0.25g (1 tablet) of ethidium bromide (C<sub>21</sub>H<sub>20</sub>BrN<sub>3</sub>) in 750 mL of MS water. Heat to 40 - 60C and stir until totally dissolved (**Caution:** Ethidium bromide is **TOXIC**. When handling, avoid contact with skin and do not breathe dust.) .....
  - b. Add 10 mL of neutral detergent, Triton X-100, and stir until totally dissolved. Adjust volume to 1 Liter with MS water .....
  - c. Store refrigerated (0 - 4.4C) in airtight, light-proof container for no longer than 90 days .....
  - d. Container labeled with date prepared and expiration date .....  
 Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_

**WORKING SOLUTIONS**

6. **Dye/Buffer Solution** .....
  - a. Dilute 1 part of Stock Dye/Buffer solution with 9 parts of MS water .....

- b. Protect from light and use within 21 days .....
- c. Date prep. \_\_\_\_\_ Exp. Date .....
7. **Rinse Solution** .....
  - a. Add 20 mL of alkaline detergent, RBS-35, per liter of MS water and mix .....
  - b. Use within 7 days .....
  - c. Date prep. \_\_\_\_\_ Exp. Date .....
8. **Optionally use manufacturer's reagent kits and instructions** .....
9. **All dye/buffer and rinsing solutions labeled with date prepared and expiration date** .....

**START UP**

10. **Cell Counter** .....
  - a. Check that the amount of dye/buffer solution (item 6) and rinse solution (item 7) in the supply containers is of sufficient volume for the number of samples to be run .....
  - 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. Coil temperature between 67 - 73C .....
  - g. Run MS water at least 3 times; reading must be zero (0) on every test .....
  - h. **IF ANY PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** .....
  - i. Records maintained on all parameters each time instrument is used .....
11. **Milk Standards** .....
  - a. Commercially prepared: \_\_\_\_\_  
 Lot# \_\_\_\_\_ Date Rcd. \_\_\_\_\_
    1. Four standards in ranges 100K - 200K, 300K - 500K, 600K - 800K and 900K - 1.2M .....
    2. Do DMSCC in triplicate on each standard in set and average counts, records maintained .....
    3. DMSCC check performed in rotation by all certified analysts .....
    4. Standards used within one week .....
  - 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 - 7.2C) of standards as received .....
    4. Maintain copies of all correspondence regarding problems .....
  - c. Laboratory prepared (weekly) .....

ELECTRONIC SOMATIC CELL COUNT
Bentley Somacount 150/300/500
(Unless other wise stated all tolerances ±5%)

- 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
5. Do DMSCC in triplicate on each standard prepared and average counts, records maintained
6. DMSCC check performed in rotation by all certified analysts
d. Hourly Control Sample (instrument drift check)
1. Use one of the standards (items 11a or b) in the 500-800K range, run in triplicate and determine average
2. Optionally, prepare sufficient control/sample 500-800K range, run in triplicate and determine average

PROCEDURE

- 12. Testing Standards (each time instrument used)
a. Heat standards to 37 - 42C (using a temperature control), and read within 30 minutes of reaching temperature, used once and then discarded, i.e., do not re-use
b. Mix by inverting at least 2x, test standards within 3 minutes
c. Run the standards in triplicate and average the counts for each level, records maintained
d. Each standard's average must be within 10% of the DMSCC (item 11) for that level, except within 15% for 100 - 200K standard, records maintained
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), records maintained
f. THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING
13. Testing samples
a. Heat samples to 37 - 42C (using a temperature control) and read within 30 minutes of reaching temperature; samples must not be re-used and must be discarded after use

- b. Mix by inverting at least 2x, test samples within 3 minutes
c. Samples must be tested within 10 minutes after being removed from waterbath
d. Samples must not be reused and must be discarded after use
e. Record number of cells counted for each sample
14. With continuous operation:
a. Run a standard or optionally a control/sample (item 11d) in the 500K 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 3x
c. Run zero control (as in item 10g) hourly
d. Maintain records
15. Routine maintenance
a. Perform as described in operating manual
b. Maintain records

REPORTS

- 16. Computing and Reporting of Counts
a. Count obtained x 1000 is the cell count/mL milk
b. In reporting optical somatic cell counts (ESCC/mL), record only first two left hand digits, raising second digit to next higher number when third digit is six 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 = 220)
d. If count on instrument is < 100 report count as < 100,000 ESCC/ML