DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

MILK LABORATORY EVALUATION FORM

LABORATORY	
LOCATION	LAB #
DATE	X = DEVIATION O = NOT USED U = UNDETERMINED NA = NOT APPLICABLE

ELECTRONIC SOMATIC CELL COUNT
Foss 250/300/360/400
[Unless otherwise stated all tolerances are ±5%]

1. Laboratory Requirements (see CP, item 33 & 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 ($K_2Cr_2O_7$) ..... _____
- 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 sub-samples 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 for DMSCC ..... _____

APPARATUS

2. See Cultural Procedures, items 1 - 5 ..... _____

3. Automated Electronic Somatic Cell Counters ..... _____
- a. **Fossomatic 250** _____ ..... _____
- b. **Fossomatic 300** _____ ..... _____
- c. **Fossomatic 360** _____ ..... _____
- d. **Fossomatic 400** _____ ..... _____

4. Water Bath ..... _____
- a. Circulating and thermostatically controlled to 37 - 42C ..... _____

REAGENTS

5. Stock Dye/Buffer Solution ..... _____
- a. Dissolve 2.5g (or number of tablets specified by manufacturer) ethidium bromide ($C_{21}H_{20}BrN_3$) in 1 liter MS water (caution TOXIC, use gloves when handling and do not breath dust), heat to 40 - 60C and mix to dissolve ..... _____
- b. Add 400g tripotassium citrate monohydrate ($C_6H_5O_7K_3 \cdot H_2O$), 14.5g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$), and 4 liters MS water, heat to 40 - 60C 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 - 4.4C) in airtight, light-proof container for no longer than 90 days ..... _____
- f. Date prep. _____ Exp. Date _____ ..... _____

6. Stock Detergent Solution ..... _____
- a. Dissolve 10 mL neutral detergent, Triton X-100 in 1 liter of MS water and heat 40 - 60C to complete solution ..... _____
- b. Store refrigerated (0 - 4.4C) in airtight, container for no longer than 30 days ..... _____
- c. Date prep. _____ Exp. Date _____ ..... _____

7. Ammonium Hydroxide (NH_2OH) Solution, reagent Grade, 25% ..... _____

8. All stock dye/buffer and detergent solutions labeled with date prepared and expiration date ..... _____

WORKING SOLUTIONS

9. Dye/Buffer Solution ..... _____
- a. Dilute 1 L dye/buffer stock solution (item 5) with 9 L MS water ..... _____
- b. Protect from light and use within 21 days ..... _____
- c. Date prep. _____ Exp. Date _____ ..... _____

10. Rinsing Solution (use within 7 days) ..... _____
- a. Add 10 mL of stock neutral detergent stock solution (item 6) and 25 mL of ammonium hydroxide solution (item 7) and suspend to 10 L with MS water ..... _____
- b. Date prep. _____ Exp. Date _____ ..... _____

11. Optionally, use manufacturer's reagent kits and instructions specific for each instrument ..... _____

12. All working dye/buffer and rinsing solutions labeled with date prepared and expiration date ..... _____

START UP

13. Cell Counter ..... _____
- a. Check that the amount of dye/buffer solution (item 9) and rinsing (cleaning) solution (item 10) in instrument supply containers is of sufficient volume for the number of samples to be run ..... _____
- b. Solutions not used beyond expiration date(s) ..... _____
- c. Turn on power and cycle at least six times ..... _____
- d. Perform a zero check before starting any measurements, within acceptable limits, single counts up to 5 and mean up to 3 ..... _____
- e. **IF ANY ABOVE PARAMETERS ARE WRONG, CORRECT BEFORE PROCEEDING** ..... _____
- f. Records maintained on all parameters each time instrument is used ..... _____

14. 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 ..... _____

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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) .....
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, used within one week .....
Date prep. _____ Exp. Date _____ .....
5. Do DMSCC in triplicate on each standard 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 14a 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

15. 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, place in rack and put onto automatic track, run within 10 minutes .....
c. Run the standards six times and average the counts for each level, records maintained .....
d. Each standard's average must be within 10% of the DMSCC (item 14) for that level, except within 15% for 100K - 200K standard, records maintained .....
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)**, records maintained .....
f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** .....

16. Testing Samples .....
a. Heat samples to 37 - 42C (using a temperature control) and read within 30 minutes of reaching temperature; samples must not be reused and must be discarded after use .....
b. Mix by inverting at least 2x, place in rack and put onto automatic track, run within 10 minutes of reaching the testing temperature .....

17. With continuous operation: .....
a. Run a standard or optionally a control/sample (item 14d) 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 6x .....
c. Run zero control (as in item 13d) .....
d. Maintain records .....

18. Routine maintenance .....
a. Perform as described in operating manual .....
b. Maintain records .....

REPORTS

19. 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 = 240) .....
b. When the second digit is even round down, delete the 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 .....