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 90/215
[Unless otherwise stated all tolerances are ±5%]

1. Laboratory Requirements (see CP, items 33 and 34) ..... _____
- a. Unpreserved samples run from 24 to 72 hours after initial collection ..... _____
- b. Samples may be run from 8 hours 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 can not be 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. Electronic Somatic Cell Counter ..... _____
- a. Automated: Fossomatic 215 _____ ..... _____
- b. Semi-Automated: Fossomatic 90 _____ ..... _____

4. Pipettor, fixed volume (Foss 90) (_____) ..... _____
- a. Calibrated to deliver 500 µL milk (see CP item 6e) ..... _____
- b. Records maintained ..... _____

5. Pipettor Tips ..... _____
- a. Disposable, replace for each sample ..... _____
- b. Reusable ..... _____
  1. Rinse in 40C MS water ..... _____
  2. Rinse in sample more than 1 time ..... _____
  3. Do not use for more than 25 samples ..... _____

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

REAGENTS

7. Stock Dye Solution, 0.1% Ethidium Bromide (caution TOXIC, use gloves when handling and do not breath dust) ..... _____
- a. Dissolve 1.0g ethidium bromide ($C_{21}H_{20}BrN_3$) in 1 liter MS water by heating to 40 - 60C ..... _____
- b. Store in light-proof, air-tight bottle no more than 60 days .... _____
- c. Date prepared _____ Exp. Date _____ ..... _____

8. Stock Rinsing Solution, 1% Triton X-100 ..... _____
- a. Dissolve 10 mL Triton X-100 in 1 liter MS water by heating to 60C ..... _____
- b. Store in air-tight container no more than 25 days ..... _____
- c. Date prepared _____ Exp. Date _____ ..... _____

9. Stock Buffer Solution, 0.025 M Potassium Hydrogen Phthalate .. _____
- a. **Automated: Fossomatic 215** ..... _____
  1. Dissolve 51.0g KH phthalate and 13.75g KOH in 10 L MS water by heating to 40 - 60C ..... _____
  2. Add 10 mL 1% Triton X-100 (item 8), store less than 7 days in airtight container ..... _____
  3. Date prep. _____ Exp. Date _____ ..... _____
- b. **Semi-automated: Fossomatic 90** ..... _____
  1. Dissolve 51.0g KH phthalate and 13.75g KOH in 10 L MS water by heating to 40 - 60C ..... _____
  2. Add 150 mL 1% Triton X-100 (item 8), store less than 7 days in airtight container ..... _____
  3 Date prep. _____ Exp. Date _____ ..... _____

10. Ammonium Hydroxide (NH_2OH) Solution, Reagent Grade, 25% ..... _____

11. All stock dye/buffer/rinsing solutions labeled with date prepared and expiration date ..... _____

WORKING SOLUTIONS

12. Working Dye Solution/Zero Control (used within 7 days) ..... _____
- a. **Foss 215**: Dilute 20 mL stock dye solution (item 7a) to 1 liter with stock buffer solution (item 9a2) ..... _____
- b. **Foss 90**: Dilute 26 mL stock dye solution (item 7a) to 2.5 liters with stock buffer solution (item 9b2) ..... _____
- c. Date prepared _____ Exp. Date _____ ..... _____

13. Working Rinsing Solution (used within 7 days) ..... _____
- a. Add 10 mL stock rinsing solution (item 8) to 25 mL of 25% NH_4OH and dilute to 10 liters with MS water ..... _____
- b. Date prepared _____ Exp. Date _____ ..... _____

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

15. All working dye and rinsing solutions labeled with date prepared and expiration date ..... _____

START UP

16. Cell Counter ..... _____
- a. Assure adequate volume of working solutions, not used beyond expiration date(s) ..... _____
- b. Turn on power and cycle at least six times ..... _____
- c. Blind count:
  1. **Foss 90**: ≤5 ..... _____
  2. **Foss 215**: <10 ..... _____
- d. **Foss 215** sample-reagent mixture temperature 50 - 60C ..... _____
- e. **Foss 90** additional checks: ..... _____
  1. Vacuum pressure setting minimum of -40 KPa ..... _____
  2. Dispenser filling time 4 - 5 seconds ..... _____

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3. Intake filling time 3 - 4 seconds .....
f. **IF ANY ABOVE PARAMETERS ARE WRONG, CORRECT BEFORE PROCEEDING** .....
g. Records maintained on all parameters .....

17. Milk Standards .....
a. Commercially prepared: _____
Lot #_____ Date Rcd. _____ .....
1. Four samples 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 samples 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-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, used within one week .....
Date prep. _____ Exp. Date _____ .....
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 17a 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

18. Testing Standards (each time instument 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. Mixing .....
1. **Foss 215**: Invert at least twice, place in rack and put on automatic track; run within 10 min .....
2. **Foss 90**: Invert 10 times, pipet 500 µL into intake chamber within 3 min .....
c. Run standards in triplicate (**Foss 90**) or 6 times (**Foss 215**) and average the counts for each level, records maintained .....
d. Each standard's average must be within 10% of the DMSCC (item 17) 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 (C_V) of 5% or less on 10 replicates **(Refer to Operating Manual)**, records maintained .....
f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** .....

19. 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. Mixing .....
1. **Foss 215**: Invert at least twice, place in rack and put on automatic track; run within 10 min .....
2. **Foss 90**: Invert 10 times, pipet 500 µL into intake chamber within 3 min .....
c. Record number of cells counted for each sample .....

20. With continuous operation:
a. Run a standard or optionally a control/sample (item 17d) 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 standard/control 3x (**Foss 90**) or 6x (**Foss 215**) .....
c. Run zero control (as in item 12) .....
d. Maintain records .....

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

REPORTS

22. Computing and Reporting 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 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 to 240) .....
b. When the 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 as < 100,000 ESCC/mL .....