

ELECTRONIC SOMATIC CELL COUNT

Foss 250/300/360/400

(Unless otherwise stated all tolerances ±5%)

- 1. Laboratory Requirements (see CP, item 33 & 34) _____
 - a. Un-preserved 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 (K₂Cr₂O₇) _____
 - 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 in DMSCC _____

APPARATUS

- 2. See Cultural Procedures, items 1-5 _____
- 3. Automated Electronic Somatic Cell Counters _____
 - a. **Fossomatic 250** _____
 - a. **Fossomatic 300** _____
 - b. **Fossomatic 360** _____
 - c. **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 deionized (DI) or 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 DI or 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 DI or 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_4OH) 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 DI or 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 DI or 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 _____

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 _____
5. Standards used by manufacturer's expiration date _____
- 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 re-used 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
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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 (item 13d)
 - d. Maintain records
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18. Routine maintenance

- a. Perform as described in operating manual
 - b. Maintain records
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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 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
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