

ELECTRONIC SOMATIC CELL COUNT

Somascope MKII/SomaScope Smart

Raw Cow Milk

[Unless otherwise stated all tolerances are $\pm 5\%$]

1. Laboratory Requirements (see Cultural Procedures, 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 ($K_2Cr_2O_7$)
- 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 in DMSCC

APPARATUS

2. See Cultural Procedures, items 1-5

3. Automated Electronic Somatic Cell Counters

- a. Somascope MKII manual
- b. Somascope MKII automatic
- c. SomaScope Smart

4. Water Bath

- a. Circulating and thermostatically controlled to 37-42C

REAGENTS

5. Staining Kits

Warning: Before using the chemicals for making up the staining solution, read the Material Safety Data Sheet (MSDS) provided in the appendix of the manual. Wear safety glasses and latex or nitrile gloves when handling the chemicals

- a. One liter concentrate kit – makes 5 L staining solution when diluted with deionized (DI) or MS water

Lot No. _____ Mfr. Exp. Date ____/____/____

1. Store at 0 - 5C and protected from light

- b. Twenty liter powder kit, consisting of three different components, mixed with 20 L DI or MS water

Lot No. _____ Mfr. Exp. Date ____/____/____

1. Staining Concentrate (powder)

- a. Store powder 0 - 5C and protected from light

2. Staining Detergent (liquid)

- a. Store at room-temperature protected from light

3. Staining Buffer (powder)

- a. Store at room-temperature protected from light

WORKING SOLUTIONS

6. Working Staining Solutions

- a. One liter Concentrate Kit

1. Add 4 L of DI or MS water

2. Mix on a magnetic stirrer at room temperature to make working stain solution

- b. Twenty liter Powder Kit _____
 - 1. Dissolve the staining buffer (item 5b3) in about 18 L of DI or MS water and stir until the powder is fully dissolved _____
 - 2. Add the staining detergent (item 5b2) to about one liter of warm (35 - 45C) DI or MS water and mix well (preferably with a magnetic stirrer) to dissolve the detergent _____
 - a. The detergent must be well dissolved, no powder residue visible (optical test) _____
 - 3. Add the detergent solution (item 6b2) to the 18 L of staining buffer (item 6b1) and mix _____
 - 4. Dissolve the Staining Concentrate (item 5b1) in 3 mL of 35 – 45C DI or MS water _____
 - a. Mix until the powder is dissolved (optical test) _____
 - b. Keep the concentrate (powder and solution) protected from strong light during preparation _____
 - 5. Add the dissolved concentrate to the approximately 19 L of buffer and add DI or MS water until the total volume is 20 L _____
- c. Store the working staining solutions up to 2 months at 0 – 5C protected from light _____

Date Prep. ___/___/___ Lab Exp. Date ___/___/___ _____
- d. Use the staining solution at room-temperature _____

Date filled ___/___/___ _____

 - 1. The contents of the staining container can be left at room temperature _____
 - 2. The contents must be used within 7 days _____
 - 3. Clean container once a month as per manufacturer's instructions _____

7. Other Working Solutions

a. Detergent Container

1. SomaScope MKII

a. Alkaline detergent – DECON 90, Contrad 70 or RBS 50

b. Fill the black detergent reservoir with about 50 mL of undiluted detergent in the Sample Preparation Unit

c. Check reservoir visually daily for volume and add more detergent as necessary

2. SomaScope Smart

a. 5% Alkaline detergent – DECON 90, Contrad 70 or RBS 50

b. Add 250 mL of detergent to DI or MS water to make 5 L of solution

c. Mix well

d. Pour the solution above into the “Cleaning” container provided with the instrument

b. Water Container(s)

1. Add 5 mL of Triton X-100 to DI or MS water to make 100 mL solution

2. Mix the above solution until the Triton X-100 is completely dissolved

3. Add the 100 mL solution above to room temperature DI or MS water to make 5 L solution

4. Mix well

5. Dispense

a. SomaScope MKII

1. Pour the solution above into the water container provided with the instrument

b. SomaScope Smart _____

1. Pour the solution above into the “Rinsing” and “Sheath Flow” containers provided with the instrument _____

START UP

8. Somatic Cell Counter _____

a. Solution Check _____

1. Check to see if the amount of staining solution (about 1.6 mL per sample), detergent and rinse solution (about 1 mL per sample) are of sufficient volume for the number of samples to be run _____
2. Solutions may not be used beyond expiration date _____

b. Instrument Initiation _____

1. SomaScope MKII _____

- a. Turn on the HBO lamp and wait 15 min _____
- b. Turn on the Sample Preparation Unit and internal computer of the SomaScope MKII _____
- c. Put the peristaltic pump in operation position _____
- d. Perform a clean sequence _____

2. SomaScope Smart _____

- a. Turn on instrument _____
- b. Put the peristaltic pumps in operation position _____
- c. Use SomaScope Smart Software to perform “Startup Wizard” _____

c. Run at least 5 times with rinse solution (item 7b), last reading must be below 5 _____

d. **IF ANY PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** _____

e. Records maintained on all parameters each time instrument is used _____

9. 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, records maintained
- 3. DMSCC check performed in rotation by all certified analysts
- 4. Standards used within one week

b. Certified provider: _____

Lot# _____ 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 of standards as received (must be 0 – 7.2C)
- 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, use within one week

Date prep. ___/___/___ Exp. Date ___/___/___

- 5. Perform 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 in the 500-800K range, run in triplicate and determine average _____
 - 2. Optionally, prepare sufficient control/sample of 500-800K range, run in triplicate and determine average _____

PROCEDURE

10. Testing Standards (each time instrument used) _____

- a. Heat standards to 37 - 42C (using a temperature control) and read within 30 min 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 min _____
- 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 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, records maintained _____
- f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

11. Testing samples _____

- a. Heat samples to 37 - 42C (using a temperature control) and read within 30 min of reaching temperature _____
- b. Mix by inverting at least 2x, test samples within 3 min _____
- c. Samples must be tested within 10 min after being removed from water bath _____
- d. Samples must not be reused and must be discarded after use _____
- e. Record number of cells counted for each sample _____

12. With continuous operation:

- a. Run a standard or optionally a control/sample (item 9d) 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 hourly (item 8c)
- d. Maintain records

13. Shut down procedure

- a. SomaScope MKII
 - 1. Perform a clean cycle twice
 - 2. Put instrument pipette in beaker of rinse solution
 - 3. Release peristaltic pump
 - 4. Switch off PC, SPU, and internal computer of Somascope MKII, HBO-lamp in this order
- b. SomaScope Smart
 - 1. Perform a clean cycle
 - 2. Prepare and heat 100 mL of 0.1% Triton X-100 (“Heated Water”) and 100 mL of 20% DECON-90 (“Heated Cleanser”) to 40C
 - 3. Perform “Shut Down Wizard”
 - 4. Put instrument pipette in beaker of rinse solution
 - 5. Release peristaltic pumps
 - 6. Switch off PC and SomaScope Smart

14. Routine maintenance

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

REPORTING

15. Computing and Reporting of Counts

- a. Count obtained x 1000 is the cell count/mL of milk
- b. In reporting electronic somatic cell counts (ESCC/mL), record only first two left hand digits
- 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 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