

ELECTRONIC SOMATIC CELL COUNT

**SomaScope™ MKII/SomaScope™ Smart/CombiScope
(Raw Cow Milk, Raw Sheep Milk, Raw Goat Milk and Raw Water Buffalo Milk)
IMS #16**

[Unless otherwise stated all tolerances are ±5%]

- 1. Laboratory Requirements (see Cultural Procedures (CP) items 33 & 34)** _____
- a. Un-preserved samples may be run up to 72 hours after initial collection _____
- b. Samples may be tested 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) _____

PRE-REQUISITE

- 2. Comparative Test with DMSCC (co-requisite for certification)** _____
- a. Analyst(s) certified for DMSCC _____
- b. Each analyst seeking certification for the ESCC test shall perform the comparative test) _____
 - 1. 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) _____
 - 2. Results must be evaluated by State/Federal LEO and shown to be acceptable prior to official use of test in laboratory _____
 - 3. Copy of comparison and results in QC record (or easily accessible on file in the laboratory); kept for as long as analyst is certified _____

APPARATUS

- 3. See CP items 1-4** _____
- 4. Automated Electronic Somatic Cell Counters** _____
 - a. SomaScope MKII manual _____
 - b. SomaScope MKII automatic _____
 - c. SomaScope Smart _____
 - d. CombiScope _____
- 5. Water Bath** _____
 - a. Circulating and thermostatically controlled to 37-42°C _____

REAGENTS

6. Reagents

- a. One liter Concentrate Kit Lot #: _____ Exp. Date: _____ _____
- b. Twenty liter Powder Kit Lot #: _____ Exp. Date: _____ _____
1. Staining Concentrate Lot #: _____ Exp. Date: _____ _____
2. Staining Detergent Lot #: _____ Exp. Date: _____ _____
3. Staining Buffer Lot #: _____ Exp. Date: _____ _____

7. Preparation of Reagents

- a. Working Stain Solution: Mix one liter Concentrate Kit (item 6.a) with 4 L of DI or MS water; mix on a magnetic stirrer at room temperature _____
- b. Twenty liter Powder Kit _____
1. Dissolve the staining buffer (item 6.b.3) in approximately 18 L of DI or MS water and stir until the powder is fully dissolved _____
2. Add the staining detergent (item 6.b.2) to approximately one liter of warm (35-45°C) DI or MS water and mix well (preferably with a magnetic stirrer) to dissolve the detergent. The detergent must be well dissolved, no powder residue visible _____
3. Add the detergent solution (item 6.b.2) to the 18 L of staining buffer (item 7.b.1) and mix _____
4. Dissolve the Staining Concentrate (item 6.b.1) in 3 mL of 35-45°C DI or MS water. Mix until the powder is dissolved. Keep the concentrate (powder and solution) protected from strong light during preparation _____
5. Add the dissolved concentrate to buffer (item 7.b.3). Add DI or MS water to make 20 L _____
- c. Store the working staining solutions up to 2 months at 0-5°C protected from light _____
- Lab Prep. Date: _____ Lab Exp. Date: _____ _____
- d. Use the staining solution at room-temperature _____
1. The contents of the staining container can be left at room temperature _____
2. The contents must be used within 7 days _____
- Date Filled: _____ Lab Exp. Date: _____ _____

3. Clean container once a month as per manufacturer's instructions _____

8. Other Solutions _____

a. Detergent Container _____

1. SomaScope MKII _____

- a. Alkaline detergent – DECON 90, Contrad 70 or RBS 50 _____
- b. Fill the black detergent reservoir with approximately 50 mL of undiluted detergent in the Sample Preparation Unit _____
- c. Check that the volume of detergent solution in the reservoir is sufficient for the number of samples to be tested _____

2. SomaScope Smart/CombiScope _____

- 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 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 above into the water container provided with the instrument _____

b. SomaScope Smart _____

1. Pour the above into the "Rinsing" and "Sheath Flow" containers provided with the instrument _____

c. CombiScope _____

1. Pour the solution above into the "Triton Water" containers provided with the instrument _____

9. All Solutions Labeled with Date Prepared and Expiration Date _____

START UP

10. Cell Counter _____

- a. Check that the volume of staining, detergent and rinse solutions in the supply containers is sufficient for the number of samples to be tested _____
- b. Solutions not used beyond expiration date(s) _____
- c. Initiate instrument _____
- d. Perform a blank check: Test the rinse solution (item 8.b) at least 5 times; the last reading must be <5 _____
- e. **IF ANY ABOVE PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** _____
- f. Maintain records 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. Perform DMSCC in triplicate on each standard in set and average counts; maintain records _____
3. Perform DMSCC check in rotation by all certified analysts _____
4. Standards used within one week _____

Lab Exp. Date: _____

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 of standards as received (must be 0.0-7.5°C) _____

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 _____

Lab Prep. Date: _____ Lab Exp. Date: _____

5. Perform DMSCC in triplicate on each standard prepared and average counts; maintain records _____

6. Perform DMSCC check in rotation by all certified analysts _____

d. Hourly Control Sample (instrument drift check) _____

1. Use one of the standards (items 11.a, b or c) in the 600-800K range, test in triplicate and determine average _____

2. Optionally, prepare sufficient control/sample of 600-800K range; test in triplicate and determine average _____

PROCEDURE

12. Testing Standards (each time instrument used) _____

a. Heat standards to 37-42°C (using a temperature control) and read within 30 min of reaching temperature, use once and discard; i.e., do not re-use _____

b. Mix by inverting at least 2x, test standards within 3 min _____

c. Test the standards in triplicate and average the counts for each level; maintain records _____

d. Each standard's average must be within 10% of the DMSCC (item 11) for that level, except within 15% for 100-200K standard; maintain records _____

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**); maintain records _____

f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

13. Testing Samples _____

- a. Heat samples to 37-42°C (using a temperature control) and read within 30 min of reaching temperature _____
- b. Test samples within 10 min after removal from water bath _____
- c. Mix by inverting at least 2x, test samples within 3 min _____
- d. Record number of cells counted for each sample _____

14. With Continuous Operation: _____

- a. Run zero control (item 10.c) hourly _____
- b. Test a standard or optionally a control/sample (item 11.d) in the 600K to 800K range hourly in triplicate and determine the average, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average) _____
- c. Maintain records _____

15. Routine Maintenance _____

- a. Maintain records _____

REPORTING

16. Computing and Reporting of 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 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 to 220) _____
- d. If count on instrument is < 100 report count as < 100,000 ESCC/mL _____
- e. If goat milk is over the regulatory limit, follow confirmation procedure in PMO _____