ELECTRONIC SOMATIC CELL COUNT

SomaScope™ MKII/SomaScope™ Smart/CombiScope
(Raw Commingled Cow, Goat, Sheep, Water Buffalo and Camel 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 ($K_2Cr_2O_7$) _____

2. Comparative Test with DMSCC
[NOT required as a co-requisite for certification of analysts in laboratories purchasing standards from a CERTIFIED provider (item 11.b)] _____

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 the FDA/LPET LEO or 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 _____

c. Required for laboratories preparing in house standards or using commercially prepared standards (items 11.a and c) and for those testing goat or camel milk _____

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 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 _____

   6. Failed standards shall be verified with DMSCC _____

      a. If no analysts certified for DMSCC then a new set of standards is required _____

      b. Do not continue with official testing until the new standard(s) test(s) in range _____

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; 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; 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.d) 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 or camel milk is over the regulatory limit, follow confirmation procedure in PMO _____