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 (K₂Cr₂O₇)

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
         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 (K₂Cr₂O₇)

   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; 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