

**DIRECT MICROSCOPIC SOMATIC CELL COUNT**  
**(Raw Commingled Cow, Goat, Sheep, Water Buffalo Milk)**  
**IMS #12**

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

**SAMPLES**

**1. Laboratory Requirements (See Cultural Procedures [CP] items 33 & 34)** \_\_\_\_\_

- a. Unpreserved samples may be tested 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-dio. (Bronopol™) or 0.05% potassium Dichromate ( $K_2Cr_2O_7$ ) \_\_\_\_\_

**APPARATUS**

**2. See CP, items 1-4** \_\_\_\_\_

- a. Functional fume hood, face velocity 100 ft/min \_\_\_\_\_
  - 1. Check annually, maintain records, and tag unit \_\_\_\_\_

**3. Microscope Slides, Clean (see item 18), 2.54 x 7.62 cm** \_\_\_\_\_

- a. 11.28 mm diameter areas delineated \_\_\_\_\_
- b. Optionally, with center marks on sides of delineated area \_\_\_\_\_
- c. Optionally, 5.08 x 7.62 or 5.08 x 11.43 cm with 11.28 mm delineated areas \_\_\_\_\_

**4. Pipetting Apparatus** \_\_\_\_\_

- a. Metal Syringe: ( \_\_\_\_\_ ) \_\_\_\_\_
  - 1. Suitable for rapid and convenient transfer of 0.01 mL of milk \_\_\_\_\_
  - 2. Check accuracy as specified in CP item 6.e to deliver  $0.0103 \pm 0.0005$  g (average of 10 consecutive weighings with milk) \_\_\_\_\_  
Avg. Wt.: \_\_\_\_\_ Date: \_\_\_\_\_
  - 3. Syringe etched with identification (imprinted serial number acceptable) and tag with accuracy check date \_\_\_\_\_
- b. Micropipettor, with appropriate tips: ( \_\_\_\_\_ ) \_\_\_\_\_
  - 1. Suitable for rapid and convenient transfer of 0.01 mL of milk \_\_\_\_\_

2. Check accuracy as specified in CP item 6.e to deliver  $0.0103 \pm 0.0005\text{g}$  (average of 10 consecutive weighings with milk) \_\_\_\_\_

a. If using Artel PCS, see CP item 6.e.4 \_\_\_\_\_

Avg. Wt.: \_\_\_\_\_ Date: \_\_\_\_\_ \_\_\_\_\_

3. Micropipettor etched with identification (imprinted serial number acceptable); tag with accuracy check date \_\_\_\_\_

c. Maintain records of accuracy check(s) \_\_\_\_\_

**5. Dissecting Needle, Bent Point** \_\_\_\_\_

a. Suitable for spreading milk film \_\_\_\_\_

**6. Drying Device, Slide Drier or Incubator** \_\_\_\_\_

a. Clean, dust-free, level surface \_\_\_\_\_

b. Regulate heat source at 40-45°C \_\_\_\_\_

1. Monitor temperature with temperature measuring device \_\_\_\_\_

**7. Forceps or Slide Holder** \_\_\_\_\_

a. Required for dipping and holding slides \_\_\_\_\_

**8. Staining Jars or Trays** \_\_\_\_\_

a. With tight fitting covers \_\_\_\_\_

b. Convenient size for holding solvents and stains \_\_\_\_\_

**9. Slide Storage** \_\_\_\_\_

a. Clean, dust-free insect-proof boxes, cases or files \_\_\_\_\_

**10. Microscope Type:** \_\_\_\_\_

a. Binocular with 1.8 mm oil immersion objective, rack and pinion sub-stage, condenser with iris diaphragm \_\_\_\_\_

b. Oculars, 10X (12X or 12.5X), Huygenian or wide-field \_\_\_\_\_

c. Optics provide a Single Strip Factor of 6070 or smaller \_\_\_\_\_

1. Each analyst measures field diameter and calculates SSF annually, round to three significant figures \_\_\_\_\_

2. Calculation of Single Strip Factor \_\_\_\_\_

- a. Using a stage micrometer (item 11), measure field diameter (D) of oil immersion objective lens in mm \_\_\_\_\_

D = \_\_\_\_\_ mm \_\_\_\_\_

- b. Compute SSF with formula: \_\_\_\_\_

$$SSF = 10,000 / (11.28 \times D)$$
 \_\_\_\_\_

SSF is \_\_\_\_\_

d. Mechanical Stage \_\_\_\_\_

1. Suitable for examination of slides, smooth action, does not drift, allows proper tracking of smears \_\_\_\_\_

e. Microscope Lamp, provides adequate illumination \_\_\_\_\_

**11. Stage Micrometer Ruled with 0.1 and 0.01 mm Divisions** \_\_\_\_\_

**12. Hand Tally, accurate** \_\_\_\_\_

**MATERIALS**

**13. Immersion Oil** \_\_\_\_\_

- a. Refractive index 1.51-1.52 at 20°C \_\_\_\_\_

**14. Levowitz-Weber Modification of the Newman-Lampert Stain** \_\_\_\_\_

- a. Slowly add 0.6 g certified methylene blue chloride to 52 mL of 95% ethyl alcohol and 44 mL of tetrachloroethane (reagent grade) in a 200 mL flask and swirl to dissolve \_\_\_\_\_

- b. When making stain, use gloves and prepare in fume hood (tetrachloroethane is TOXIC) \_\_\_\_\_

- c. Let stand for 12-24 hours at 4.5-7.5°C \_\_\_\_\_

- d. Filter through Whatman No. 42 filter paper or equivalent \_\_\_\_\_

- e. Add 4 mL of glacial acetic acid \_\_\_\_\_

- f. Store in a clean, tightly closed container (traces of water or solvent may cause problems with this stain) \_\_\_\_\_

- g. Or, Commercially prepared (xylene or tetrachloroethane) \_\_\_\_\_

Brand: \_\_\_\_\_ Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

**15. Canadian Formula Stain**

- a. Commercially prepared (xylene or tetrachloroethane)

Brand: \_\_\_\_\_ Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

**16. Alternate Methylene Blue Stain**

- a. Prepare as in item 14 with reagents:

- Combine: 0.5 g cert. methylene blue chloride  
56 mL 95% ethyl alcohol  
40 mL xylene  
4 mL glacial acetic acid

**17. Pyronin Y-Methyl Green Stain for Goat or Sheep Milk**

- a. Carnoy's fixative

- Combine: 60 mL chloroform  
20 mL glacial acetic acid  
120 mL 100% ethyl alcohol

- Or, Commercially Prepared

Brand: \_\_\_\_\_ Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

- b. Pyronin Y-methyl green stain

- Combine: 1.0 g Pyronin Y  
0.56 g methyl green  
196 mL water

- Filter through Whatman No. 1 paper before use

- Stain is light sensitive; store in brown bottle

- Or, Commercially Prepared

Brand: \_\_\_\_\_ Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

**18. Slides, Cleaning**

- a. Physically clean

- b. New slides may be cleaned by soaking in strong cleaning solution

- c. Rinse thoroughly in flowing water 10-15 sec and DI water

- d. Used slides may be soaked in hot detergent or wetting agent until all residues are removed; rinse as above

e. Air or heat dry with minimal exposure to dust, insects, etc. and store dry \_\_\_\_\_

f. Or, store slides in alcohol and flame just before use \_\_\_\_\_

## PROCEDURE

### 19. Slide Identification \_\_\_\_\_

a. Legibly and indelibly identify each sample area on margin of slide \_\_\_\_\_

### 20. Sample Agitation \_\_\_\_\_

a. Mix samples or subsamples by shaking 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting; use within 3 min (samples must be in appropriate containers to allow the use of vortexing) \_\_\_\_\_

b. Optionally, warm high fat samples to 40°C for no longer than 10 min prior to testing (discard after testing). Mix as in item 20.a \_\_\_\_\_

### 21. Sample Measurement and Smear Preparation (Metal Syringe) \_\_\_\_\_

a. Before use and between successive samples, rinse syringe 2-3 times in clean, 25-35°C water \_\_\_\_\_

b. Before transferring test portion to slide, insert syringe not over 1 cm below surface of milk and repeatedly rinse (avoid foam and bubbles) \_\_\_\_\_

c. Holding tip beneath surface, rinse syringe three times with milk, then fully depress and release plunger and withdraw test portion \_\_\_\_\_

d. With clean paper tissue, remove excess milk from exterior of tip (with syringe tip up, wipe downward away from tip) \_\_\_\_\_

e. Holding instrument vertical, place tip near center of area for smear, touch the slide with the tip and expel the test portion \_\_\_\_\_

1. With plunger still fully depressed, touch off once against a dry spot \_\_\_\_\_

2. Do not release plunger until after touching off and removing tip from slide \_\_\_\_\_

3. Spread milk with point of bent needle point (item 5); not hockey stick style \_\_\_\_\_

4. Wipe needle dry between samples on tissue \_\_\_\_\_

f. When preparing multiple smears, complete steps 21.a through 21.e.4 before starting the next smear \_\_\_\_\_

g. After spreading test portion, dry smears at 40-45°C within 5 min on level surface (item 6) \_\_\_\_\_

- h. To prevent smears from cracking and peeling from slide during staining, do not heat too rapidly \_\_\_\_\_
- i. Protect smears and slides from damage until read \_\_\_\_\_

**22. Metal Syringe Cleaning** \_\_\_\_\_

- a. Do not allow residues to dry on instrument \_\_\_\_\_
- b. Immediately after use, carefully disassemble and clean syringe \_\_\_\_\_
- c. Do not remove spring unless necessary \_\_\_\_\_
- d. Use only soap-less detergents and/or fat solvents sparingly as needed \_\_\_\_\_
- e. Clean all residues from measuring tube circulating detergent with bulb on delivery end \_\_\_\_\_
- f. Clean piston with dry paper tissue \_\_\_\_\_

**23. Sample Measurement and Smear Preparation (Micropipettor)** \_\_\_\_\_

- a. Use new tip for each sample \_\_\_\_\_
- b. Depress plunger and insert tip below surface, fully release plunger slowly, remove tip from sample and touch off to neck of sample container (avoid foam and bubbles) \_\_\_\_\_
- c. If necessary, remove excess milk from exterior of tip by wiping away from the tip with clean paper tissue \_\_\_\_\_
- d. Holding instrument vertical, place tip near center of area for smear, expel test portion \_\_\_\_\_
  - 1. Move to dry spot on slide \_\_\_\_\_
    - a. If pipettor only has one (1) stop, touch off \_\_\_\_\_
    - b. If pipettor has two (2) stops, depress plunger to second stop, touch off \_\_\_\_\_
- e. Spread milk with point of bent needle point (item 5); not hockey stick style \_\_\_\_\_
- f. Wipe needle dry between samples on tissue \_\_\_\_\_
- g. When preparing multiple smears, complete steps 23.a through 23.f before starting the next smear \_\_\_\_\_
- h. After spreading test portion, dry smears at 40-45°C within 5 min on level surface (item 6) \_\_\_\_\_

- i. To prevent smears from cracking and peeling from slide during staining, do not heat too rapidly \_\_\_\_\_
- j. Protect smears and slides from damage until read \_\_\_\_\_

**24. Staining Films** \_\_\_\_\_

- a. Levowitz-Weber and Methylene Blue Stains \_\_\_\_\_
  - 1. Use ventilated hood for steps 2-4 \_\_\_\_\_
  - 2. Submerge or flood slides in stain for 2 min (timer used) \_\_\_\_\_
  - 3. Drain off excess stain by resting edge of slide on absorbent paper \_\_\_\_\_
  - 4. Dry thoroughly (air dry or use cool forced air) \_\_\_\_\_
  - 5. Dip dry stained slides in 3 changes of tap water at 35-45°C \_\_\_\_\_
  - 6. Drain and air dry slides before examining smears \_\_\_\_\_
- b. Pyronin Y-Methyl Green Stain (New York Modification) \_\_\_\_\_

Note: Stain is light sensitive and must be protected from overexposure to light

  - 1. Slide is run through the following staining scheme \_\_\_\_\_
    - Carnoy's fixative 5 min
    - 50% Ethanol 1 min
    - 30% Ethanol 1 min
    - Water 1 min
    - Stain 6 min
    - N-Butyl alcohol flush briefly
    - Xylene flush briefly
  - a. Optionally, if smears will not adhere to slides: \_\_\_\_\_
    - 1. Allow slide to dry, (approx.10 min) protected from overexposure to light, after Carnoy's fixative step but before the 50% ethanol step OR \_\_\_\_\_
    - 2. Allow slide to dry (approx.10 min) protected from overexposure to light, after stain step but before flushing with N-Butyl alcohol \_\_\_\_\_
  - 2. Cells stain blue or blue-green; RNA and background stain pink \_\_\_\_\_

**25. Examination** \_\_\_\_\_

- a. Adjust microscope lamp to provide maximal optical resolution \_\_\_\_\_
- b. Locate edge of smear to be read using low power \_\_\_\_\_
- c. Place 1 drop immersion oil on smear \_\_\_\_\_

- d. Carefully lower oil immersion lens \_\_\_\_\_
- e. Focus and locate center of edge of area and begin counting cells \_\_\_\_\_
- f. Count all cells in field wide strip across diameter of a single smear, focusing up and down as necessary (horizontally or vertically) \_\_\_\_\_
- g. Identifying and counting somatic cells \_\_\_\_\_
  - 1. Cells possess a nucleus that stains dark blue for cow, water buffalo and other Merocrine (bovine) secretory systems \_\_\_\_\_
  - 2. Cells possess a nucleus that stains blue or blue-green for goats, sheep and other Apocrine (caprine) secretory systems \_\_\_\_\_
  - 3. Count those cells (nuclear masses) within the strip and also those cells that are touching one edge of the strip, but not the other edge \_\_\_\_\_
  - 4. Fragments are counted only if more than 50% of the nuclear material is visible \_\_\_\_\_
  - 5. Count clusters of cells as one unless nuclear unit(s) is clearly separated: focus up and down to ensure there are no bridges connecting nuclear masses \_\_\_\_\_
  - 6. If in doubt, do not count \_\_\_\_\_
- h. After examination of each smear record strip count \_\_\_\_\_
- i. Conduct monthly comparative counting between analysts (see SPC Agar (2400a) item 18 or Petrifilm (2400a-5) item 17) \_\_\_\_\_

## REPORTS

### 26. Records and Reporting \_\_\_\_\_

- a. Maintain record of strip count for each smear examined \_\_\_\_\_
- b. Compute DMSCC/mL, multiply number of cells counted (strip count) by the SSF (item 10.c.2.b) \_\_\_\_\_
- c. Report somatic cell counts as DMSCC/mL, record only first two left hand digits, round as necessary \_\_\_\_\_
  - 1. If the third digit is 5 round the second number using the following rules \_\_\_\_\_
    - a. When the second digit is odd round up (odd up, 235 to 240) \_\_\_\_\_
    - b. When the second digit is even round down (even down, 225 to 220) \_\_\_\_\_