

DIRECT MICROSCOPIC SOMATIC CELL COUNT
(Raw Commingled Cow, Goat, Sheep, Water Buffalo and Camel 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.4 to deliver 0.0103 ± 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.4 to deliver 0.0103 ±0.0005g (average of 10 consecutive weighings with milk)

a. If using Artel PCS, see CP item 6.e.5

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 _____

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:

- | | | |
|-------------|-------------------------------|-------|
| 1. Combine: | Cert. Methylene Blue Chloride | 0.5 g |
| | 95% Ethyl Alcohol | 56 mL |
| | Xylene | 40 mL |
| | Glacial Acetic Acid | 4 mL |

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

- a. Carnoy's fixative

- | | | |
|-------------|---------------------|--------|
| 1. Combine: | Chloroform | 60 mL |
| | Glacial Acetic Acid | 20 mL |
| | 100% Ethyl Alcohol | 120 mL |

2. Or, Commercially Prepared

Brand: _____ Lot #: _____ Exp. Date: _____

- b. Pyronin Y-methyl green stain

- | | | |
|-------------|--------------|--------|
| 1. Combine: | Pyronin Y | 1.0 g |
| | Methyl Green | 0.56 g |
| | Water | 196 mL |

2. Filter through Whatman No. 1 paper before use

3. Stain is light sensitive; store in brown bottle

4. 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

- 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 tap 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 by 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 24.a.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
DI or MS 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 ovine _____
 - 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 plate count procedure FDA/NCIMS 2400 forms, Identifying Counting Errors) _____

REPORTS

26. Records and Reporting _____

- a. 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) _____
- d. Maintain records _____