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

[Unless otherwise stated all tolerances are ±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^{TM}) 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 ± 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 ± 0.0005g (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 40-45ºC _____

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 x 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 20ºC _____

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 4.5-7.5ºC _____

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: 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 _____

1. Combine: 60 mL chloroform
20 mL glacial acetic acid
120 mL 100% ethyl alcohol _____

2. Or, Commercially Prepared _____

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

b. Pyronin Y-methyl green stain _____

1. Combine: 1.0 g Pyronin Y
0.56 g methyl green
196 mL water _____

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 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 40ºC 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, 25-35ºC 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 40-45ºC 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 40-45ºC 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 35-45ºC _____

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) _____