DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

**MILK LABORATORY EVALUATION FORM**

| LABORATORY | |
|---|---|
| LOCATION | LAB # |
| DATE | X = DEVIATION O = NOT USED U = UNDETERMINED NA = NOT APPLICABLE |

# DIRECT MICROSCOPIC SOMATIC CELL COUNT

**[Unless otherwise stated all tolerances are ±5%]**

## SAMPLES

**1. Laboratory Requirements (See CP, item 33 & 34)** ..... _____

## APPARATUS

**2. See Cultural Procedures, items 1-4** ..... _____
- a. Functional fume hood, face velocity 100 cu. ft/min ..... _____
  - 1. Checked annually, records maintained, unit tagged ..... _____

**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 cm delineated areas ..... _____

**4. Syringe** ..... _____
- a. Metal (_____) ..... _____
  - 1. Suitable for rapid and convenient transfer of 0.01 mL of milk ..... _____
  - 2. Calibrated quarterly to deliver 0.0103±0.0005g (average of 10 consecutive weighings with milk) ..... _____
    Avg. Wt. _____ Date _____ ..... _____
  - 3. Syringe etched with identification, and tagged with calibration date ..... _____
- b. Micropipettor, with appropriate tips (_____) ..... _____
  - 1. Suitable for rapid and convenient transfer of 0.01 mL of milk ..... _____
  - 2. Calibrated quarterly to deliver 0.0103±0.0005g (average of 10 consecutive weighings with milk) ..... _____
    Avg. Wt. _____ Date _____ ..... _____
  - 3. Syringe etched with identification and tagged with calibration date ..... _____
- c. Records of syringe (metal or micro) calibration maintained ..... _____

**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. Heat source regulated at 40-45C ..... _____
  - 1. Temperature monitored with thermometer ..... _____

**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 20C ..... _____

**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 tetrachlorethane (reagent grade) in a 200 mL flask and swirl to dissolve ..... _____
- b. When making stain, use gloves and prepare in fume hood (tetrachlorethane is TOXIC) ..... _____
- c. Let stand for 12-24 hr at 4.4-7.2C ..... _____
- 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 tetrachlorethane) ..... _____
  Brand _____ Lot No _____ ..... _____

**15. Canadian Formula Stain** ..... _____
- a. Commercially prepared (xylene or tetrachlorethane) ..... _____
  Brand _____ Lot No _____ ..... _____

**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 Milk** ..... _____
- a. Carnoy's fixative ..... _____
  - 1. Combine: 60 mL chloroform ..... _____
    20 mL glacial acetic acid ..... _____
    120 mL 100% ethyl alcohol ..... _____
- 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 ..... _____

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# DIRECT MICROSCOPIC SOMATIC CELL COUNT

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**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 MS water .....
- d. Used slides may be soaked in hot detergent or wetting agent until all residues are removed, rinsed 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 by shaking 25 times in 7 sec with 1 ft movement, sample removed within 3 minutes .....
- b. Optional: Warm high fat samples to 40C for no longer than 10 minutes prior to testing (discard after testing) .....

**21. Sample Measurement and Smear Preparation (Metal Syringe)** .....
- a. Before use and between successive samples, rinse syringe 2 - 3 times in clean, 25-35C water .....
- b. Before transferring test portion to slide, dip tip of syringe not over 1 cm below surface (excluding foam) of milk and repeatedly rinse .....
- 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 or cloth, 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 or towel .....
- f. After spreading test portion, dry smears at 40-45C within 5 min on level surface (see item 6) .....
- g. To prevent smears from cracking and peeling from slide during staining, do not heat too rapidly .....
- h. 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 or cloth .....

**23. Sample Measurement and Smear Preparation (Micropipettor)** .....
- a. Use clean tip for each sample .....
- b. Depress plunger and dip tip not over 1 cm below surface (excluding foam) of well-mixed milk, fully release plunger slowly, remove tip from sample and dispel back to sample, re-insert tip and fully release plunger and withdraw test portion, touch off to dry area of sample container .....
- c. If necessary, remove excess milk from exterior of tip by wiping away from the tip with clean paper tissue or cloth .....
- d. Holding instrument vertical, place tip near center of area for smear, expel test portion and touch off once to dry spot .....
- e. Spread milk with point of bent needle point (item 5), not hockey stick style .....
- f. Wipe needle dry between samples on tissue or towel .....
- g. After spreading test portion, dry smears at 40-45C within 5 min on level surface (see 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

**24. Staining Films** .....
- a. Levowitz-Weber and Methylene Blue Stains .....
  1. Use ventilated hood for steps 2-4 .....
  2. Submerge or flood slides with fixed, dried smears 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 35-45C .....
  6. Drain and air dry slides before examining smears .....
- b. Pyronin Y-Methyl Green Stain (New York Modification) .....
  1. Slide is run through the following staining scheme .....

| | | |
|---|---|---|
| Carnoy's fixative | 5 min | ..... |
| 50% Ethanol | 1 min | ..... |
| 30% Ethanol | 1 min | ..... |
| $H_20$ | 1 min | ..... |
| Stain | 6 min | ..... |
| N-Butyl alcohol | flush briefly | ..... |
| Xylene | flush briefly | ..... |

  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 .....

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g. Identifying and counting somatic cells ..... _____
   1. Cells possess a nucleus stained dark blue (bovine) or blue or blue-green (caprine) ..... _____
   2. Cells generally 8 microns or larger (bovine; caprine may be smaller); do not count cells less than 4 microns; fragments counted only if more than 50% of nuclear material visible ..... _____
   3. Cluster of cells counted as one unless nuclear unit(s) are clearly separated; focus up and down to ensure that there are no bridges connecting nuclear masses ..... _____
   4. Count cells touching only top or bottom half of strip ..... _____
   5. If in doubt, do not count ..... _____

h. After examination of each smear record strip count ..... _____

i. Conduct monthly comparative counting between analysts (refer to SPC item 19) ..... _____

**26. Slide Storage** ..... _____

a. Remove oil by dipping in xylene (or equivalent), 15-20 sec ..... _____

b. Air dry ..... _____

c. Place in suitable storage (item 9) ..... _____

## REPORTS

**27. 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) ..... _____