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

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory sample requirements (see CP item 33 & 34) .... _____

STANDARD PLATE AND COLIFORM METHODS
DILUTING SAMPLES

2. Work Area .... _____
   a. Level plating bench not in direct sunlight .... _____
   b. Sanitized immediately before start of plating .... _____

3. Selecting Dilutions .... _____
   a. Standard Plate Count .... _____
      1. Plate two decimal dilutions per sample .... _____
      2. Select dilutions to yield one plate with 25-250 colonies ..... _____
         a. Raw milk is normally diluted to 1:100 and 1:1000 .... _____
         b. Finished products are normally diluted to 1:10 and
           1:100 .... _____
         c. The above are general guidelines and may have to be
           adjusted on a case by case basis (dilutions below 1:10
           not required) .... _____
   b. Coliform Counts .... _____
      1. For milk samples, 1 mL direct and/or decimal dilutions .... _____
      2. For all other products, distribute 10 mL of a 1:10 dilution
         among three plates, generally high fat and viscous
         products .... _____

4. Identifying Plates .... _____
   a. Label each plate with sample identification and dilution .... _____
   b. Arrange plates in order before preparation of dilutions .... _____

5. Sample Agitation .... _____
   a. When appropriate, wipe top of unopened containers with
      sterile, ethyl alcohol-saturated cloth .... _____
   b. Before removal of any portion, thoroughly mix contents of
      each container .... _____
      1. Shake raw and processed sample containers (approx ¾
         full) 25 times in 7 sec with 1 ft movement .... _____
      2. Invert filled retail container 25 times, each inversion a
         complete down and up motion .... _____
   c. Remove test portion within 3 min of sample agitation .... _____

6. Sample Measurement, pipets .... _____
   a. Use separate sterile pipets for the initial transfers from each
      container .... _____
      1. Pipets in pipet container adjusted without touching the
         pipets .... _____
   b. Pipet tip not dragged over exposed exterior of pipets in
      container .... _____
   c. Pipet not dragged across lip or neck of sample container .... _____
   d. Pipet not inserted more than 2.5 cm (1") below sample
      surface (foam avoided if possible) .... _____
   e. Draw test portion above pipet graduation mark and remove
      pipet from liquid .... _____

      1. Pipet aid used, mouth pipetting not permitted
         (_____) .... _____
   f. Adjust test volume to mark with lower side of pipet in
      contact with inside of sample container (above the sample
      surface) .... _____
   g. Drainage complete, excess liquid not adhering to pipet .... _____
   h. Release test portion to petri dish (tip in contact with plate,
      45º angle) or dilution blank (with lower side of pipet in
      contact with neck of dilution blank, or dry area above buffer
      where appropriate) with column drain of 2-4 sec .... _____
   i. Blow out last drop of undiluted sample from pipet using
      pipet aid .... _____
      1. Blow out away from main part of sample in plate, do not
         make bubbles .... _____
   j. Pipets discarded into disinfectant, or if disposable into
      biohazard bags or containers to be sterilized .... _____

7. Sample Measurement, pipettors
   (_____) .... _____
   a. Each day before use, vigorously depress plunger 10x to
      redistribute lubrication and assure smooth operation
      (mechanical pipettors) .... _____
   b. Before each use examine pipettor to assure that no liquid is
      expelled from the pipettor nose-cone (contaminated), if
      fouling is detected do not use until cleaned as per manufac-
      turer recommendation .... _____
   c. Use separate sterile tip for the initial transfers from each
      container .... _____
   d. Depress plunger to first stop (mechanical pipettors) .... _____
   e. Tip/barrel not dragged across lip or neck of sample con-
      tainer, and pipettor barrel not allowed within sample
      container .... _____
   f. Tip not inserted more than 1 cm below sample surface
      (foam avoided if possible) .... _____
   g. With pipettor vertical **slowly** and completely release plunger
      (for electronic pipettors follow manufacturer instructions) ...... _____
   h. Remove tip from sample and depress plunger completely,
      re-insert tip into sample and repeat steps f and g, and then
      remove tip from liquid .... _____
   i. Touch tip off to inside of sample container above the sample
      surface, excess liquid not adhering to tip (do not lay pipettor
      down once sample is drawn up, use vertical rack if necessary) .... _____
   j. Release test portion to petri dish (tip in contact with plate)
      by slowly depressing plunger to first stop allowing about 1
      or 2 seconds for complete drainage .... _____
   k. Move tip to a dry spot on plate .... _____
      1. If pipettor only has one (1) stop touch off .... _____
      2. If pipettor has two (2) stops, depress plunger to second
         stop and touch off .... _____

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

l. Or, dispense test portion to dilution blank (tip in contact neck of dilution blank, or dry area above buffer where appropriate) by slowly depressing plunger to first stop .... _____

m. If pipettor has two (2) stops, depress plunger to second stop ..... _____

n. Tips discarded into disinfectant, biohazard bags or containers to be sterilized .... _____

8. Dilution Agitation .... _____

a. Before removal of any portion, shake each dilution bottle 25 times in 7 sec with a 1 ft movement..................................... _____

b. Optionally, use approved mechanical shaker for 15 sec .... _____

c. Remove test portion within 3 min of dilution agitation .... _____

9. Dilution Measurement, pipets .... _____

a. Use separate sterile pipets for the initial transfers from each container .... _____

1. Pipets in pipet container adjusted without touching the pipets .... _____

b. Pipet tip not dragged over exposed exterior of pipets in container .... _____

c. Pipet not dragged across lip or neck of dilution blank........... _____

d. Pipet not inserted more than 2.5 cm (1") below dilution surface .... _____

e. Draw dilution portion above pipet graduation mark and remove pipet from liquid .... _____

1. Pipet aid used, mouth pipetting not permitted (_____) .... _____

f. Adjust dilution volume to mark with lower side of pipet in contact with inside of dilution blank neck .... _____

g. Drainage complete, excess liquid not adhering to pipet .... _____

h. Gently lift cover of petri dish just high enough to insert pipet

i. Hold pipet at 45° angle to dish with tip touching dish (or dilution blank neck) .... _____

j. Release dilution portion to dish (or dilution blank) with tip in contact with the bottom of the dish (or dilution blank neck, or dry area above buffer where appropriate) with column drain of 2-4 sec .... _____

k. Touch pipet tip once against dry spot on dish bottom (or dilution blank neck) .... _____

l. When measuring 0.1 mL, do not re-touch dry area .... _____

m. Pipets discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized .... _____

10. Dilution Measurement, pipettors (_____) .... _____

a. Use separate sterile tip for the initial transfers from each container .... _____

b. Depress plunger to first stop (mechanical pipettors) .... _____

c. Tip/barrel not dragged across lip or neck of dilution blank, and pipettor barrel not allowed within dilution blank............. _____

d. Tip not inserted more than 1 cm below dilution surface .... _____

e. With pipettor vertical **slowly** and completely release plunger (for electronic pipettors follow manufacturer instructions) ..... _____

f. Remove tip from dilution and depress plunger completely, re-insert tip into dilution and repeat steps d and e, and then remove tip from liquid .... _____

g. Touch tip off to inside of dilution blank neck or dry area above buffer where appropriate, excess liquid not adhering to tip .... _____

h. Gently lift cover of petri dish just high enough to insert tip .... _____

i. Hold pipettor nearly vertical to dish with tip touching dish .... _____

j. Release test portion to petri dish (tip in contact with plate) by slowly depressing plunger to first stop .... _____

k. Move tip to a dry spot on plate .... _____

1. If pipettor only has one (1) stop touch off .... _____

2. If pipettor has two (2) stops, depress plunger to second stop and touch off .... _____

l. Tips discarded into disinfectant, biohazard bags/containers or into spent dilution blanks to be sterilized .... _____

11. Samples Other than Milk .... _____

a. Weigh 11g aseptically into dilution blank .... _____

b. Use dilution blanks heated to 40-45C .... _____

12. Dry Milk Samples .... _____

a. Weigh 11g aseptically into dilution blank heated to 40-45C ..... _____

1. Use standard dilution blank .... _____

2. Or, 2.0% sodium citrate blank (pH<8.0) for relatively insoluble sample (not to be used with Petrifilm) .... _____

b. Wet sample completely with gentle agitation (invert) .... _____

c. Let soak 2 min, then shake 25 times in 7 sec with l ft movement, use within 3 minutes of agitation .... _____

PLATING

13. Plating .... _____

a. Melt agar quickly in boiling water, flowing steam not under pressure, or microwave oven (use extreme caution when microwaving) .... _____

b. Avoid prolonged exposure to high temperatures during and after melting, establish lab protocol .... _____

c. Do not melt more than will be used within 3 hours .... _____

d. Do not melt agar more than once .... _____

e. Promptly cool melted agar to 45±1C .... _____

1. Record temperature with other control information .... _____

f. Temperature control used for each test medium type .... _____

1. Contains medium identical to type being used .... _____

2. In container identical to that being used .... _____

3. Undergoes *same* heat treatment and cooling as test medium .... _____

g. Select number of samples in any series so that all will be plated within 20 min (pref ≤ 10) after diluting first sample .... _____

h. After depositing test portions, promptly pour 10-12 mL medium into each plate of series, or 15-20 mL for > 1 mL portion/plate or where agar weight loss is a problem that can not be corrected by other actions (documentation must be kept to indicate that this is a routine practice) .... _____

i. Lift cover of petri dish just high enough to pour medium ..... _____

j. As each plate is poured thoroughly and evenly mix medium and test portion in petri dish .... _____

1. Multiple plates may be poured and mixed, however, plates may not be stacked prior to mixing .... _____

k. Allow to solidify within 10 min on level surface .... _____

l. For dry milk sample, overlay plate with 3-5 mL PCA .... _____

m. For coliform count, overlay plate with 3-4 mL VRB .... _____

n. Invert and incubate within 10 min of medium solidification .... _____

LABORATORY	LAB #	LOCATION	DATE

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

CONTROLS

14. Controls .... _____
   a. Check sterility of dilution blanks, medium, petri dishes, and pipets used for each group of samples (AM and PM) .... _____
   b. Expose a poured plate with cover completely removed or pre-hydrated Petrifilm Aerobic Count (PAC) film (both wet surfaces completely exposed) to air for 15 min during plating, AM and PM .... _____
      1. The air control plate must be the first plate poured immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side) .... _____
      2. If count > 15, take and note corrective actions .... _____
      3. For PAC films see item 45.b.7 .... _____
   c. Records maintained .... _____
   d. Include information on bench, work sheet or report sheet(s) .... _____

INCUBATION

15. Incubation .... _____
   a. Incubate SPC plates at 32±1C for 48±3 hours (dry milk for 72±3 hours) and incubate coliform plates at 32±1C for 24±2 hours .... _____
   b. Stack plates no more than 6 high .... _____
   c. Arrange stacks so each is at least 1" from adjacent stacks and from incubator surfaces .... _____
   d. Place stacks directly over each other on successive shelves ..... _____

COUNTING COLONIES

16. Counting Aids .... _____
   a. Count colonies with aid of magnification under uniform and properly controlled artificial illumination with a hand tally .... _____

17. Recording Standard Plate Count .... _____
   a. After incubating plates, promptly count all colonies on selected plates .... _____
   b. Where impossible to count at once, store plates at 0-4.4C for not longer than 24 hr (avoid as a routine practice) .... _____
   c. Record dilutions used and number of colonies on each plate counted .... _____
   d. Record results of sterility and control tests .... _____
   e. When possible, select spreader free plates with 25-250 colonies and count all colonies including those of pinpoint size .... _____
      1. Use higher magnification if necessary to distinguish colonies from foreign matter .... _____
      2. Examine edge of petri plates for colonies .... _____
   f. If consecutive plates yield 25-250 colonies, count all colonies on plate(s) from both dilutions .... _____
   g. Spreaders .... _____
      1. Count colonies on representative portion only when colonies are well distributed and area covered or re-pressed does not exceed 25% of plate .... _____
      2. Do not count if repressed growth area > 25% of plate area
      3. When spreaders must be counted, count each as a single colony .... _____

      4. Count chains/spreaders from separate sources as separate colonies .... _____
      5. If 5% of plates are more than ¼ covered by spreaders, take immediate steps to eliminate and resolve problem .... _____
   h. If there is no 25-250 colony plate, use plate having nearest to 25 or 250 colonies .... _____
   i. If plates from all dilutions exceed 250 colonies, estimate counts as follows .... _____
      1. Count colonies in portions representative of distribution and estimate total .... _____
      2. Where there are < 10 colonies/sq cm, count colonies in 12 squares, selecting 6 consecutive squares horizontally across the plate and six consecutive squares at right angles .... _____
      3. When there are 10 or more colonies/sq cm, count 4 representative squares .... _____
      4. Multiply average number colonies/sq cm by area of plate in sq cm .... _____
   j. If plates yield < 25 colonies each, record actual number in lowest dilution .... _____
   k. If all plates from a sample show no colonies, record count as 0 .... _____

18. Coliform Count .... _____
   a. After incubating plates, promptly count colonies .... _____
   b. Where impossible to count at once, store plates at 0-4.4C for not longer than 24 hr (avoid as a routine practice) .... _____
   c. Dark red colonies measuring 0.5 mm or more in diameter on agar plates are considered coliforms in plates containing ≤ 154 colonies .... _____
   d. On crowded plates, coliform colonies may be atypical; count and confirm presence of lactose fermentors .... _____
   e. Confirmation of colonies .... _____
      1. Pick 10% up to 10 representative colonies per plate with relative percentages of each colony type and inoculate into brilliant green lactose bile broth; incubate 24-48 hr at 32±1C as appropriate .... _____
      2. Presence of any gas in a BGB tube constitutes a con-firmed test .... _____
      3. Record the number of picked colonies and the number of colonies that produced gas (if necessary calculate % confirmed and multiply by total number of colonies) .... _____
   f. If no colonies appear on plate(s), record count as 0 .... _____
   g. If there are 1-154 colonies on a plate, record number counted .... _____
   h. If > 154 colonies develop in highest dilution plate, record number as > 150 .... _____
   i. When multiple plates of a dilution are used, sum counts of plates .... _____

19. Personal Errors .... _____
   a. Avoid inaccurate counting due to carelessness, fatigue, or impaired vision .... _____
   b. Discover cause and correct if unable to duplicate your own counts on the same plate .... _____
   c. Perform monthly counting .... _____

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

1. If 3 or more analysts use the RpSm method, see current SMEDP, records maintained .... _____
2. If less than three analysts, comparative counts agree $\leq$ 8% for the same analyst and $\leq$ 10% between two analysts, records maintained .... _____

REPORTS

20. **Computing and Reporting Counts** .... _____
   a. Multiply number of colonies (or estimated number if necessary) by the reciprocal of the dilution .... _____
   b. If consecutive dilutions yield 25-250 colonies, compute count using formula below (see current SMEDP) .... _____

   $N = \Sigma C / [(1 \times n_1) + (0.1 \times n_2)]d$

   Where, N = number of colonies per milliliter or gram
   ΣC = sum of all colonies on all plates counted
   n_1 = number of plates in lower dilution counted
   n_2 = number of plates in next highest dilution counted
   d = dilution from which the first counts were obtained

   Example: 1:100 = 244 colonies 1:1,000 = 28 colonies

   N = $(244 + 28)/[(1 \times 1) + (0.1 \times 1)]0.01$
   = 272 / [1.1]0.01
   = 272 / 0.011
   = 24,727 [25,000 (reported)]

   Note: In the NCIMS Program the denominator will always be 0.11 for 1:10 dilutions and 0.011 for 1:100 dilutions

   c. Report SPC and coliform counts only if inhibitors are *not* detected .... _____
   d. Report computed count as Standard Plate Count/mL or /g (SPC/mL or SPC/g) when taken from plate(s) in the 25-250 range ..... _____
   e. Report count as Coliform Count (confirmed)/mL or /g when taken from plate(s) in the l-l54 range .... _____
   f. If no colonies appear on SPC plates, report as < 25 times the reciprocal of the dilution and report as estimated .... _____
   g. If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as estimated .... _____
   h. Report SPC plate counts of 0 to 24 as < 25 times the reciprocal of the dilution and report as estimated .... _____
   i. When colonies on SPC plates exceed 100/sq cm, compute count by multiplying 100 x dilution factor x area of plate in sq cm and report as > computed count estimated .... _____
   j. Computed counts from SPC plates outside the 25-250 range are reported as Estimated SPC (ESPC) .... _____
   k. Counts from coliform plates > 154 are reported as > 150 Estimated Coliform Count (ECC) .... _____
   l. If for any reason, an entire plate is not counted, the computed count is reported as Estimated .... _____
   m. Report only first two left-hand digits .... _____
      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) .... _____
   n. If all plates from a sample have excessive spreader growth, report as spreaders (SPR), or are known to contain inhibitor(s) report as growth inhibitors (GI) .... _____
   o. If a laboratory accident renders a plate uncountable, report as laboratory accident (LA) .... _____

PLATE LOOP COUNT METHOD
APPARATUS

21. **Loop 0.001 mL** .... _____
   a. True circle, welded I.D. 1.45±0.06 mm, calibrated to contain 0.001 mL, made of appropriate wire .... _____
   b. Loop fits over a No. 54 but not a No. 53 twist drill bits (lab must have set), checked monthly, records maintained .... _____
   c. Modified by making a 30° bend 3-4 mm from loop, compare to template before use .... _____
   d. Opposite end of wire kinked in several places .... _____
22. **Hypodermic Needle, Luer-Lok** .... _____
   a. 13 gauge (sawed off 24-36 mm from the point where the barrel enters the hub) .... _____
   b. Kinked end of loop wire shank inserted into needle until bend is 12-14 mm from end of barrel, compare to template before use .... _____
23. **Cornwall Continuous Pipetting Outfit** .... _____
   a. Consisting of metal holder, Cornwall Luer-Lok syringe and filling outfit .... _____
   b. Syringe, 2 mL capacity, adjusted to deliver 1.0 mL .... _____
      1. Calibrated by checking ten 1 mL discharges (10 mL) using a 10 mL Class A graduate cylinder each day of use, records maintained .... _____
   c. With Luer-Lok of needle attached to Luer-Lok fitting of syringe .... _____

PREPARATION

24. **Heat Treatment of Pipetting Equipment** .... _____
   a. Sterilize assembled outfit wrapped in kraft paper or in a closed container by autoclaving at 120±1C for 15 min .... _____
25. **Assembly of Complete Apparatus for Use** .... _____
   a. Place end of rubber supply tube (attached to syringe) in sterile dilution buffer blank and depress syringe plunger several times to pump buffer into syringe .... _____
   b. Briefly flame loop and allow to cool 15 sec .... _____
   c. Discharge several 1 mL portions to waste, then discharge 1 mL portion of buffer into instrument control plate .... _____

PROCEDURE

26. **Comparative Test with SPC** .... _____
   a. Comparisons done by each analyst performing test .... _____
      1. Comparison is valid only if done using similar plate count methods, i.e. SPC agar with pipets (or pipettors) to SPC agar with the PLC device or Petrifilm with pipets (or pipettors) to Petrifilm with the PLC device. Mixing methods is not permissible .... _____
      2. Results must be shown to be acceptable prior to official use of test in laboratory .... _____

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
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b. Copy of comparison and results in QC record (or easily accessible file in laboratory) .... _____

27. Identifying Plates (as item 4) .... _____

28. Sample Agitation (as item 5) .... _____

29. Inoculating Plates .... _____

    a. Dip loop into each sample (avoiding foam) to bend in shank and withdraw vertically from surface three times in 3 sec with uniform movement of 2.5 cm .... _____

    b. Raise cover of petri dish (just high enough to insert loop), insert loop and depress plunger causing sterile dilution buffer to flow across charged loop washing measured 0.001 mL of sample into dish .... _____

    c. Do not depress plunger so rapidly that buffer fails to flow across loop .... _____

30. Plating .... _____

    a. As described in item 13 or 44 .... _____

    b. Pour plates with 12-15 mL agar .... _____

CONTROLS

31. Controls .... _____

    a. See item 14 .... _____

    b. Initial rinse control, see item 25c and 29c .... _____

    c. Determine if loop is free rinsing by preparing a rinse control plate after every 20 samples plated .... _____

    d. After all samples have been run discharge a final rinse to a control plate .... _____

INCUBATION

32. Incubation (see item 15) .... _____

    a. 48±3 hr at 32±1C .... _____

COUNTING COLONIES

33. Counting Aids (see item 16 or 47b) .... _____

34. Recording Plate Loop Counts (see item 17 or 48) .... _____

35. Personal Errors (see item 19 or 49) .... _____

REPORTS

36. Reporting Counts .... _____

    a. See item 20 or 50 .... _____

    b. If 0 to 24 colonies on plate report as < 25,000 Estimated Plate Loop Count/mL (EPLC/mL) or if Petrifilm used, EPPLC/mL .... _____

    c. If count is between 25 and 250, report count as PLC/mL or PPLC/mL .... _____

    d. If colony count is > 250, report as EPLC/mL or EPPLC/mL ..... _____

    e. When colonies exceed 100/sq cm, compute count by multiplying 100 x dilution factor x area of plate in sq cm and report as > computed count estimated .... _____

PETRIFILM AEROBIC COUNT METHOD
APPARATUS

37. Petrifilm Aerobic Count (PAC) Films .... _____

38. Plastic Spreader .... _____

    a. Provided with Petrifilm films, concave (ridge) side used .... _____

PROCEDURE

39. Identifying Films (as item 4) .... _____

40. Sample Agitation (as item 5) .... _____

41. Sample Measurement (as item 6&7) .... _____

42. Dilution Agitation (as item 8) .... _____

43. Dilution Measurement (as item 9&10) .... _____

44. Procedure .... _____

    a. Place the film on a level surface .... _____

    b. Lift the top film and deposit 1 mL of sample or dilution onto the center of the base film, touching off the last drop .... _____

        1. Deposit samples with pipet (since only 1 mL samples can be used; 10 fold dilution will have to be made) .... _____

        2. Or, deposit samples with pipettor (capable of making a 1:10 dilution in the tip) .... _____

        3. Or, deposit sample with PLC apparatus (item 29) .... _____

    c. Carefully *drop* the top film onto the inoculum .... _____

    d. Place the plastic spreader with the ridge side down (item 38) on the top film over the sample and press down gently on the center of the spreader to distribute inoculum to the circular ridge of the spreader .... _____

    e. Leave film undisturbed for 1 min for gel solidification .... _____

    f. Incubate within 10 min of solidification .... _____

45. Controls .... _____

    a. See item 14 above except for air plates .... _____

    b. Air plates .... _____

        1. Inoculate PAC film with dilution buffer (1 mL) .... _____

        2. Drop film down onto the dilution buffer and spread as described in item 44d above .... _____

        3. Leave film undisturbed for 1 minute for solidification of gel .... _____

        4. The film must be the first one prepared immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side) ..... _____

        5. Roll top film back and away from bottom film and expose film for 15 min .... _____

        6. After the 15 min roll top film back down and incubate with other films as usual .... _____

        7. Incubated, exposed films should contain ≤ 10 colonies, if count > 10, take and note corrective actions .... _____

INCUBATION

46. Incubation .... _____

    a. Place films in horizontal position, clear side-up .... _____

    b. Stack films no more than 20 high .... _____

    c. Incubate 48±3 hr at 32±1C .... _____

COUNTING COLONIES

47. Counting PAC Films .... _____

    a. See item 16, or .... _____

    b. Optionally, enumerate using Petrifilm Information Management System (PIMS) .... _____

        1. Refer to manufacturer's instructions for set-up and operation information .... _____

        2. Controls .... _____

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

a. Store control cards in a clean, dry and enclosed container .... _____

b. Scan and record control card result prior to the start of and at the end of each operation period .... _____

c. Scan and record control card result a minimum of once per each hour of operation .... _____

d. Control card result must fall in the 92 to 108 range, if outside of this range an alarm will sound to alert the operator of a failure .... _____

   1. If alarm sounds, inspect card for defects, if defect(s) are observed replace control card, scan and report result of new card .... _____

   2. Do not proceed unless control card gives acceptable result, seek technical assistance .... _____

e. Maintain records .... _____

48. Petrifilm Count .... _____

a. Count all colonies stained various shades of red, even those outside the circular indentation left by the spreader .... _____

b. See item 17 .... _____

c. Select spreader free films with 25-250 colonies and count all red colonies .... _____

d. If films from all dilutions yield < 25 colonies each, record actual number in lowest dilution .... _____

e. If all films from a sample show no colonies, record count as 0 ..... _____

f. If films from all dilutions exceed 250 colonies, estimate (as per manufacturer specification)

49. Personal Errors .... _____

a. See item 19, or .... _____

b. If using PIMS unit analysts must perform monthly visual counts comparing to PIMS results .... _____

   1. If one analyst, count must be ≤ 10% between visual and PIMS result .... _____

   2. If two or more analysts, use RpSm method (see current SMEDP) using PIMS result as an analyst count .... _____

REPORTS

50. Reporting Counts .... _____

a. See item 20 .... _____

b. If the count is between 25 and 250, report count as Petrifilm Aerobic count/mL (PAC/mL) .... _____

c. If count is 0 to 24, report as < 25x reciprocal of the dilution as Estimated PAC/mL (EPAC/mL) .... _____

d. If count is > 250, report as EPAC/mL .... _____

e. When colonies exceed 100/sq cm, compute count by multiplying 100 x dilution factor x 20 sq cm and report as > computed count estimated .... _____

PETRIFILM COLIFORM COUNT METHOD
APPARATUS

51. Petrifilm Coliform Count (PCC) Films .... _____

52. Plastic Spreader .... _____

a. Provided with Petrifilm films, smooth, flat side used .... _____

PROCEDURE

53. Selecting Dilutions .... _____

a. For milk samples, 1 mL direct and/or decimal dilutions .... _____

b. For other milk products use 1/10 dilution, must plate 10 mL, i.e., use 10 PCC films or see 64d .... _____

54. Identifying Films (as item 4) .... _____

55. Sample Agitation (as item 5) .... _____

56. Sample Measurement (as item 6 & 7) .... _____

57. Procedure .... _____

a. Place films on level surface .... _____

b. Lift top film and deposit 1 mL of sample above the center of the base film, touching off the last drop .... _____

c. Carefully **roll** the top film into the inoculum, avoid trapping air bubbles .... _____

d. Place the plastic spreader with the flat side down (item 52) on the top film and press down gently on the center of the spreader to distribute inoculum over growth area .... _____

e. Leave films undisturbed for 1 min for gel solidification .... _____

f. Incubate films within 10 min of solidification .... _____

INCUBATION

58. Incubation .... _____

a. Place films in horizontal position, clear side up .... _____

b. Stack films no more than 20 high .... _____

c. Incubate 24±2 hr at 32±1C .... _____

COUNTING COLONIES

59. Counting Aids (see item 16) .... _____

60. Petrifilm Count .... _____

a. Count only red colonies having 1 or more gas bubbles within 1 colony diameter .... _____

b. Colonies with gas bubbles are confirmed, no other testing is required .... _____

REPORTS

61. Reporting Counts .... _____

a. See item 20 .... _____

b. If the count is between 1 and 154, report count as Petrifilm Coliform count/mL (PCC/mL) .... _____

c. If count is 0, report as < 1 Estimated PCC/mL (EPCC/mL) .... _____

d. If count is > 154, report as > 150 EPCC/mL .... _____

PETRIFILM HIGH-SENSITIVITY COLIFORM COUNT METHOD
APPARATUS

62. Petrifilm High-Sensitivity Coliform Count (HSCC) Films .... _____

63. Plastic Spreader for HSCC Films .... _____

PROCEDURE

64. Selecting Dilutions .... _____

a. For milk samples, apply 5 mL direct and/or make decimal dilutions .... _____

b. 1:5 minimum dilution required for: chocolate milk, cottage cheese, dip, evaporated milk, frozen yogurt, heavy and light cream, ice cream, sour cream, sweetened condensed milk and/or decimal dilutions .... _____

c. 1:10 minimum dilution required for: butter, buttermilk, cheese, dry dairy products, yogurt and/or decimal dilutions .... _____

STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS
[Unless otherwise stated all tolerances are ±5%]

    d. 1:10 dilutions of milk or milk products test 10 mL (5 mL on two films) .... _____

65. Identifying Films (as item 4) .... _____

66. Sample Agitation (as item 5) .... _____

67. Sample Measurement (as item 6 & 7) .... _____

68. Procedure .... _____

    a. Place film on level surface .... _____

    b. Lift top film and deposit 5 mL of sample or dilution just above the center of the bottom film, touching off the last drop .... _____

    c. Carefully **roll** the top film onto the sample gently to prevent pushing the inoculum off the film and to avoid trapping air bubbles .... _____

    d. Place the plastic spreader (item 63) on the top film over the inoculum

    e. Distribute sample with a gentle downward pressure on the handle of the spreader to distribute inoculum to the circular ridge of the spreader .... _____

    f. Leave film undisturbed for 2-5 min for gel to solidify .... _____

    g. Incubate within 10 min of solidification .... _____

INCUBATION

69. Incubation (see item 58) .... _____

    a. Stack films no more than 10 high .... _____

COUNTING COLONIES

70. Counting Aids (see item 16) .... _____

71. Petrifilm Count (see items 18 & 60) .... _____

REPORTS

72. Reporting Counts .... _____

    a. See items 20 and 61 .... _____

    b. On 5 mL direct films report:

        1. 1 to 4 colonies as < 1 coliform/mL or gm .... _____

        2. 5 colonies as 1 coliform/mL or gm .... _____

    3. > 5 colonies as 1 coliform for every 5 colonies counted, rounding up to the next number if not even multiples of 5 (ex. 11=3 coliforms/mL or gm) .... _____

    c. 5 mL of 1:5 dilution provides a 1:1 sensitivity .... _____

    d. 5 mL of 1:10 dilution provides a sensitivity of 2 coliforms/mL or gm, run 1:10 dilutions in duplicate to get a sensitivity of 1 coliform/mL or gm as required by the PMO .... _____