PEEL PLATE® AEROBIC, COLIFORM AND HIGH VOLUME SENSITIVITY COLIFORM METHODS IMS # 6 (PPAC), IMS # 18 (PPEC, PPECHVS)

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory Sample Requirements (CP items 33 & 34) _____
[For inhibitor testing requirements, refer to Section 6 of the PMO]

MATERIALS AND APPARATUS

2. Peel Plate Aerobic Count (PPAC), Peel Plate Total Coliform (PPEC, *E. coli* and Total Coliform) and Peel Plate Total Coliform High Volume Sensitivity (PPECHVS) _____

PROCEDURE

3. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

4. Selecting Dilutions _____

a. Aerobic Count, PPAC

1. Plate two decimal dilutions per sample _____

2. Select dilutions that would be expected 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 _____

3. PPAC not performed on cultured or acidified products _____

b. Total Coliform, PPEC _____

1. For pasteurized fluid milk samples (except chocolate), 1 mL direct and/or decimal dilutions, as appropriate _____

2. For chocolate milk samples (flavored milk optional), distribute 2 mL of a 1:2 dilution (1 part sample and 1 part diluent) among two (2) PPEC plates, mL per plate _____

3. For samples other than milk (item 11) distribute 10 mL of a 1:10 dilution (1 part sample and 9 parts diluent) among ten (10) PPEC plates, 1 mL per plate or use PPECHVS plates (item 4.c) _____

4. For PPEC performed on cultured product containing active Lactic Acid Bacteria (LAB), e.g. cottage cheese _____

a. Prepare diluent with 0.2% sodium bisulfite _____

1. Use commercially available sterile 20% solution of sodium bisulfate, or prepare a 20% solution of sodium bisulfite and filter or heat sterilize. Keep refrigerated. Add 1 mL of sterile 20% sodium bisulfite to 99 mL sterile dilution buffer _____

2. Alternatively, add 0.2 g sodium bisulfite to 99 mL dilution buffer or MS water and sterilize _____

a. Homogenize 1:10 dilution (1 part sample and 9 parts sodium bisulfite diluent) _____

b. Distribute homogenate among ten (10) PPEC plates, 1 mL per plate or use PPECHVS plates _____

c. High Volume Sensitivity Coliform, PPECHVS _____

1. At least a 1:10 minimum dilution required for: evaporated milk, heavy and light cream, sweetened condensed milk, sour cream, and sour cream based dips and eggnog (flavored milk optional) _____

2. For cultured product containing active LAB, e.g. cottage cheese

a. Prepare diluent with 0.2% sodium bisulfite _____

1. Use commercially available sterile 20% solution of sodium bisulfate, or prepare a 20% solution of sodium bisulfite and filter or heat sterilize. Keep refrigerated. Add 1 mL of sterile 20% sodium bisulfite to 99 mL sterile dilution buffer _____

2. Alternatively, add 0.2 g sodium bisulfate to 99 mL dilution Buffer or MS water and sterilize _____

b. Homogenize 1:10 dilution (1 part sample and 9 parts diluent) _____

2. Test 10 mL of homogenate 1:10 dilution (5 mL on 2 plates) _____

d. For acidified products, it is not necessary to adjust pH because of buffering capacity in the Peel Plate test. The pH range of a rehydrated test may be checked with different acidified products using pH paper to verify that the rehydrated tests will be in range. Document for product type and dispose test with pH paper contact _____

1. PPEC – pH range 6.6 to 7.2 _____

2. PPECHVS – pH range 6.5 to 7.5 _____

3. Refer to manufacturer's instructions for list of low pH products that may require adjustment before plating _____

5. Identifying Peel Plate Tests _____

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

b. Label each plate with sample or control identification and dilution _____

c. Arrange plates in order before preparation of dilutions _____

CONTROLS

6. Controls (AM and PM) _____

a. Check sterility of dilution blanks, PPAC plates, and pipets/tips used for each group of samples _____

b. Expose a rehydrated PPAC plate to air during plating for 15 min _____

1. The air control plate must be the first plate set up 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) _____

a. Inoculate the center of the PPAC with 1 mL dilution buffer as described in items 9.i.1 or 10.i _____

b. Pull adhesive film off and adhere to top side of plate. Leave plate open, completely exposing rehydrated surface for 15 min; use timer _____

c. After 15 min, replace adhesive film back down as described in 9.i.2 and incubate as described in item 10.i.2 _____

2. After incubation, air plate(s) shall contain ≤ 5 colonies _____

3. Take and record corrective actions for air control plate(s) with > 5 colonies _____

a. Maintain records _____

b. Include information on bench sheet, work sheet or report sheet(s) _____

DILUTING SAMPLES

7. Sample Agitation _____

a. When appropriate, wipe top of unopened containers with sterile, ethyl alcohol-saturated cloth _____

b. Before removal of any portion or sub-samples, thoroughly mix contents of each container _____

1. Mix raw sample(s) by shaking 25 times in 7 sec with a 1 ft movement (containers approx., ¾ full) _____

2. Mix retail milk samples by inverting containers top to bottom, then bottom to top (a complete half circle or 180 degrees) without pausing, 25 times _____

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

8. Dilution Agitation _____

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

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

c. Mechanical shakers may be used only if a laboratory provides validation data on a specific unit. Data must pass validation criteria _____

PLATING

9. Sample and Dilution Measurement, Pipets _____

a. Use separate sterile pipets for the initial transfers from each container, adjust pipets in pipet container without touching the pipets _____

b. Do not drag pipet tip over exposed exterior of pipets in pipet container _____

c. Do not drag pipet across lip or neck of sample container or dilution blank _____

d. Insert pipet not more than 2.5 cm (1”) below sample surface or dilution surface (avoid foam and bubbles) _____

e. Using pipet aid, draw test portion above pipet graduation mark and remove pipet from liquid (mouth pipetting not permitted) _____

f. Adjust test volume to mark with lower side of pipet: _____

1. In contact with inside of sample container (above the sample surface) _____

2. Or, in contact with inside of dilution blank neck or area above buffer on straight-walled container _____

3. Ensure excess liquid does not adhere when pipet is removed from the sample container or dilution blank _____

g. For dilutions, dispense test portion to dilution blank (with lower side of pipet in contact with neck of dilution blank, or area above buffer on straight-walled containers) with column drain of 2-4 sec _____

h. Keeping plate flat on bench, peel back the top adhesive film (PPEC and PPAC) or lift plate top (PPECHVS) to fully expose the test plate _____

i. Deposit 1 mL (PPAC/PPEC), or 5 mL (PPECHVS) of sample or dilution keeping plate flat and pipet nearly vertical and in center of plate _____

1. Rapidly release sample or dilution portion vertically just above the center of the plate base with tip slightly above, but not in contact with base plate, with a continuous column drain of 2-4 sec _____

a. Using pipet aid, blow out last drop of undiluted sample, away from main part of sample on plate _____

b. Gently touch off pipet to dry area _____

2. PPAC/PPEC – Replace the adhesive film onto base preventing wrinkles. Apply pressure around perimeter to seal _____

3. PPECHVS – Replace the lid. Immediately lift plate from table, gently rotate plate to fully wet dry area with sample and place back on table _____

j. Leave plates undisturbed for gel solidification: _____

1. 10 sec for PPAC/PPEC _____

2. 1 min for PPECHVS _____

k. Discard pipets into disinfectant OR dispose into biohazard bags or containers to be sterilized, (using this method of disposal does not require placing into disinfectant first) _____

10. Sample & Dilution Measurements, Pipettors [for electronic pipettors, follow manufacturer instructions] Mechanical ____ Electronic ____ _____

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 manufacturer recommendation _____

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

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

e. Do not drag tip/barrel across lip or neck of sample container or dilution blank, and do not allow pipettor barrel within sample container _____

f. Insert tip approximately 0.5-1.0 mm below sample or dilution surface (avoid foam and bubbles) _____

g. With plate flat and pipettor vertical, slowly and completely release plunger on mechanical pipettor; do not lay pipettor down once sample is drawn up, use vertical rack or charging stand if necessary _____

h. Touch off lower side of tip:

1. To inside of sample container above the sample surface, excess liquid not adhering to tip _____

2. Or to the inside of dilution blank neck or area above buffer on straight-walled containers, excess liquid not adhering to tip _____

a. For dilutions, hold pipettor nearly vertical with lower side of tip touching neck of dilution blank (or area above buffer on straight-walled containers), dispense test portion to blank by slowly depressing plunger to stop (mechanical pipettor) _____

3. For two (2) stop pipettors, depress plunger to second stop with tip remaining in contact with dilution blank _____

i. Lift the top adhesive film or lid, fully exposing medium circle and keep plate flat. Deposit 1 mL (PPAC/PPEC), or 5 mL (PPECHVS) of sample or dilution keeping pipettor nearly vertical _____

1. Rapidly release sample or dilution portion within 2-4 sec vertically onto the center or just above the center of the plate with tip slightly above but not in contact with plate by slowly depressing plunger completely _____

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

b. Do not touch off pipettor tip(s) on plates _____

c. Optionally, deposit samples with pipettor capable of making a 1:10 dilution in the tip _____

2. PPAC/PPEC – Replace the adhesive film onto base preventing wrinkles. Apply pressure around perimeter to seal _____

3. PPECHVS – Replace the lid. Immediately lift plate from table, gently rotate plate to fully wet dry area with sample and place back on table _____

j. Leave plates undisturbed for gel solidification: _____

1. 10 sec for PPAC/PPEC _____

2. 1 min for PPECHVS _____

k. Discard tips into disinfectant OR dispose into biohazard bags or containers to be sterilized (using this method of disposal does not require placing into disinfectant first) _____

11. Samples other than milk _____

a. Weigh 11 g aseptically into a 99 mL dilution blank heated to 40-45°C _____

12. Dry Milk Product Samples _____

a. Weigh 11 g aseptically into a 99 mL dilution blank heated to 40-45°C _____

b. Wet sample completely with gentle inversions _____

c. Let soak a minimum of 2 min; shake 25 times in 7 sec with a 1 foot movement, use within 3 min of agitation _____

INCUBATION

13. Incubating Peel Plate Plates (see CP item 15) _____

a. Stack plates in horizontal position, clear side up _____

1. PPAC/PPEC – no more than 20 high _____

2. PPECHVS – no more than 6 high _____

b. Incubate within 10 min _____

1. PPAC for 48±3 hours at 32±1°C _____

2. PPEC and PPECHVS for 24±2 hours at 32±1°C; except when testing yogurt, incubate 48±3 hours _____

COUNTING COLONIES

14. Counting Aids (see CP item 16) _____

a. Count colonies with aid of magnification under uniform and properly controlled artificial illumination _____

b. Hand tally (see CP item 17) _____

15. Counting, Recording and Computing Aerobic Count, PPAC _____

a. After incubation count all colonies on selected plates _____

b. Where impossible to count at once, store plates at 0.0-4.5°C for not longer than 24 hours (avoid as a routine practice) _____

c. Record results of sterility and control tests _____

d. Record dilutions used and number of colonies on each plate counted _____

e. When possible, select spreader colony free plates with 25-250 colonies and count all red colonies _____

    1. Use higher magnification if necessary to distinguish colonies from foreign matter _____

    2. Examine edge of plates for colonies _____

    3. Count all colonies stained various shades of red _____

f. If consecutive plates yield 25-250 colonies, count all colonies on plates from both dilutions _____

g. Spreader colonies or plates with gel liquefaction _____

    1. Count colonies on representative portion only when colonies are well distributed and area covered, repressed or liquefied colonies do not exceed 25% of plate _____

    2. Do not count if repressed growth area or gel liquefaction >25% of plate area _____

    3. When spreader colonies must be counted, count each dark spot within the spread growth as a single colony _____

    4. Count chains/spreader colonies from separate sources as separate colonies _____

    5. If 5% of plates are more than 25% liquefied or covered by spreader colonies, take immediate steps to eliminate and resolve problem _____

h. If there is no plate yielding 25-250 colonies, use plate having nearest to 250 colonies _____

i. If plates from all dilutions exceed 250 colonies, estimate _____

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

k. If all plates from a sample show no colonies, record count as 0 _____

l. Multiply number of colonies (or estimated number if necessary) by the reciprocal of the dilution _____

1. If consecutive dilutions yield 25-250 colonies, compute count using formula below _____

$N = \Sigma C/[(1 \times n1) + (0.1 \times n2)]d$
Where, N = number of colonies per milliliter or gram
ΣC = sum of all colonies on all plates counted
n1 = number of plates in lower dilution counted
n2 = 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

16. Counting, Recording and Computing Total Coliform, PPEC and PPECHVS _____

a. After incubation count all colonies on selected plates _____

b. Where impossible to count at once, store plates at 0.0-4.5°C for not longer than 24 hours (avoid as a routine practice) _____

c. Count all colonies regardless of color or size. Red colonies are coliform producing galactosidase while blue/purple and black colonies are coliform producing the enzymes galactosidase and glucuronidase. (No further confirmation is required) _____

1. Cultured products containing LAB, e.g. yogurt, may present a red background; count distinct darker red and blue/purple colonies after 48±3 hours as coliform _____

d. If no colonies appear on plate(s), record count as 0 _____

e. If there are 1-154 colonies on a plate, record number counted _____

f. If > 154 colonies develop on highest dilution plate, record number as > 150 _____

g. When multiple plates of a dilution are used (items 4.a.2.a and 4.a.2.b), sum counts of the plates _____

h. Multiply number of colonies (or estimated number if necessary) by the reciprocal of the dilution _____

17. Identifying Counting Errors _____

a. Perform monthly counting for PPAC _____

1. With 3 or more analysts, use the RpSm method (see current SMEDP); maintain records _____

2. With two analysts, comparative counts agree within < 10%; maintain records _____

3. If only one analyst, replicate counts agree within 8% of one another; maintain records _____

REPORTING

18. Reporting (see CP item 34.b.2.d) _____
[When samples are demonstrated to contain inhibitors, no bacteria counts are reported; report as positive for inhibitors or growth inhibitors (GI)]

a. Aerobic Count, PPAC _____

1. Report computed count as Peel Plate Aerobic Count/mL or /g (PPAC/mL or PPAC/g) when taken from plate(s) in the 25-250 range _____

2. Report PPAC plate counts of 0 to 24 as < 25 times the reciprocal of the dilution and report as Estimated PPAC (EPPAC) _____

3. When colonies on PPAC plates exceed 100/sq. cm, compute count by multiplying 100 x dilution factor x 20 sq. cm and report as > computed count Estimated (EPPAC) _____

4. If computed counts from PPAC plates >250, report as Estimated PPAC (EPPAC) _____

5. If for any reason, an entire plate is not counted, the computed count is reported as Estimated (EPPAC) _____

b. Total Coliform, PPEC _____

1. Report count as Peel Plate Coliform/mL or /g (PPEC/mL or PPEC/g) when taken from plate(s) in the 1-154 range _____

a. For chocolate milk run 1:2 dilutions (1 part sample and 1 part diluent) in duplicate and sum results to detect 1 coliform/mL (1 PPEC/mL) as required by the PMO _____

2. If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as Estimated (EPPEC) _____

3. Counts from coliform plates > 154 are reported as > 150 Estimated Peel Plate Coliform Count (EPPEC) _____

c. High Sensitivity Total Coliform, PPECHVS

1. Run 1:10 dilutions in duplicate to get a sensitivity of 1 coliform/mL or g (PPECHVS) as required by the PMO _____

2. If for any reason, an entire plate is not counted, the computed count is reported as Estimated (EPPECHVS) _____

d. 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, 135 to 140) _____

b. When the second digit is even round down (even down, 125 to 120) _____

e. If all plates from a sample have excessive spreader colony growth or liquefiers, report as spreaders (SPR) or liquefiers (LIQ) _____

f. If a laboratory accident renders a plate uncountable, report as laboratory accident (LA) _____