

**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 egnog (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.,  $\frac{3}{4}$  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 \_\_\_\_\_



I. 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 \_\_\_\_\_

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

Where, N = number of colonies per milliliter or gram

$\Sigma C$  = 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

$$N = (244 + 28) / [(1 \times 1) + (0.1 \times 1)]0.01$$

$$= 272 / [1.1]0.01$$

$$= 272 / 0.011$$

$$= 24,727 [25,000 \text{ (reported)}]$$

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) \_\_\_\_\_