CHARM® PEEL PLATE® AEROBIC AND COLIFORM PROCEDURES
IMS #5c (PPAC), 20b (PPCC, PPEC, PPCCHV, PPECHV and CD Forms)

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory Sample Requirements (see Cultural Procedures [CP] items 34 & 35) [For inhibitor testing requirements, refer to Section 6 of the PMO] _____

MATERIALS AND APPARATUS

2. Peel Plate Aerobic Count (PPAC), Peel Plate Coliform Count (PPCC), Peel Plate E. coli and Coliform (PPEC), Peel Plate Coliform High Volume (PPCCHV), Peel Plate E. coli and Coliform Count High Volume (PPECHV) and Cultured Dairy (CD) Forms of Coliform tests (PPCCCD, PPECCD, PPCCCDHV and PPECCDHV) _____

PROCEDURE

3. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

4. Selecting Dilutions _____

a. 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. PPCC or PPEC _____

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

2. For chocolate milk samples (other flavored milk optional), distribute 2 mL of a 1:2 dilution (1 part sample and 1 part diluent) among two (2) PPCC/PPEC plates, 1 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) PPCC/PPEC plates, 1 mL per plate or use PPCCHV/PPECHV plates (item 4.c) _____

4. For PPCC/PPEC performed on cultured product containing active Lactic Acid Bacteria (LAB), e.g. cottage cheese, use manufacturer prepared plates for Cultured Dairy, PPCCCD or PPECCD and follow item 4.b.3 _____

   a. Or alternatively if using PPCC or PPEC plates, prepare diluent with 0.2% sodium bisulfite _____

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

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

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

      1. Mix as in item 8, or _____
      2. Vortex at highest setting for 10 seconds, or _____
      3. Blend for 2 min, or _____
      4. Stomach for 2 min _____

   c. For solid products, let settle for 30 sec. Distribute supernatant (liquid portion) of homogenate among ten (10) PPCC/PPEC plates, 1 mL per plate or use PPCCHV/PPECHV/PPCCCDHV or PPECCDHV plates _____

c. High Volume Sensitivity Coliform, PPCCHV/PPECHV/PPCCCDHV or PPECCDHV _____

   1. For evaporated milk, heavy and light cream, sweetened condensed milk, sour cream, and sour cream based dips and eggnog (flavored milk optional) prepare either a 1:5 minimum dilution or 1:10 dilution _____

   2. For cultured product containing active LAB, e.g. cottage cheese, use manufacturer prepared plates for Cultured Dairy, PPCCCDHV or PPECCDHV _____

      a. Or alternatively if using PPCCHV or PPECHV plates, prepare diluent with sodium bisulfite as in 4.b.4.a above _____

      b. Homogenize (see 4.b.4.b above) a 1:5 dilution (1 part sample and 4 parts sodium bisulfite diluent) or a 1:10 dilution (1 part sample and 9 parts diluent) _____

3. Test 1:5 dilution/homogenate (see item 4.b.4.c) by dispensing 5 mL to one plate, or test 1:10 dilution/homogenate by dispensing 5 mL to each of 2 plates (10 mL total) _____

d. For most acidified products, it is not necessary to adjust the pH due to the buffering capacity of the Peel Plate medium. The buffering capacity may be evaluated with different acidified products using Litmus paper to verify that the pH will be in the acceptable range. Document for product type and discard the plate contacted by the Litmus paper _____

1. PPCC/PPEC/PPCCCD or PPECCD – pH range 6.6 to 7.2 _____

2. PPCCHV/PPECHV/PPCCCDHV or PPECCDHV – 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 min) 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. Pull adhesive film off and adhere to top side of plate _____

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

c. Leave plate open, completely exposing rehydrated surface for 15 min; use timer _____

d. 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 1-3 sec _____

h. Keeping plate flat on bench, peel back the top adhesive film to fully expose medium _____

i. Deposit 1 mL (PPAC/PPCC/PPEC/PPCCCD/PPECCD), or 5 mL (PPECHV/PPCCHV/PPCCCDHV/PPECCDHV) of sample or dilution keeping plate flat and pipet nearly vertical above center of plate _____

1. Rapidly release sample or dilution test portion holding pipet vertically just above the center of the plate with tip slightly above, but not in contact with medium, with a continuous column drain of 1-3 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 a dry area or edge of plate _____

c. If necessary to fully wet dry medium, immediately lift plate from table and gently rotate plate to get sample across dry medium. Place plate back on table. _____

2. PPAC/PPCC/PPEC/PPCCHV/PPECHV/PPCCCD/PPECCD/PPCCCDHV/PPECCDHV – Replace the adhesive film onto base, preventing wrinkles. Apply pressure around perimeter to seal _____

j. Leave plates undisturbed for gel solidification: _____

1. 10 seconds for PPAC/PPCC/PPEC/PPCCCD/PPECCD _____

2. 1 min for PPCCHV/PPECHV/PPCCCDHV/PPECCDHV _____

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. Keeping plate flat on bench, peel back the top adhesive film (PPAC/PPCC/PPEC/PPCCHV/PPECHV/PPCCCD/PPECCD/PPCCCDHV/PPECCDHV) to fully expose medium. Deposit 1 mL (PPAC/PPCC/PPEC/PPCCCD/PPECCD) or 5 mL (PPECHV/PPCCHV/PPCCCDHV/PPECCDHV) of sample or dilution, keeping plate flat and pipet nearly vertical above center of plate _____

1. Rapidly release sample or dilution portion within 1-3 seconds vertically onto the center or just above the center of the plate with tip slightly above but not in contact with medium 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 _____

d. If necessary to fully wet dry medium, immediately lift plate from table and gently rotate plate to get sample across dry medium. Place plate back on table. _____

2. PPAC/PPCC/PPEC/PPCCHV/PPECHV/PPCCCD/PPECCD/PPCCCDHV/ PPECCDHV – Replace the adhesive film onto base, preventing wrinkles. Apply pressure around perimeter to seal _____

j. Leave plates undisturbed for gel solidification: _____

1. 10 sec for PPAC/PPCC/PPEC/PPCCCD/PPECCD _____

2. 1 min for PPCCHV/PPECHV/PPCCCDHV/PPECCDHV _____

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/PPCC/PPEC/PPCCD/PPECCD – no more than 20 high _____

2. PPCCHV/PPECHV/PPCCCDHV/PPECCDHV – no more than 12 high _____

b. Incubate within 10 min _____

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

2. PPCC/PPEC/PPCCCD/PPECCD and PPCCHV/PPECHV/PPCCCDHV/ PPECCDHV for 24±2 hours at 32±1°C; except when testing with bisulfite diluent, 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) _____

c. Optionally, count using approved Charm Peel Plate Counter (CPPC) _____

1. Test calibration prior to the start of and at the end of reading test plates _____

a. Store Calibrators in a clean, dry container, protected from light _____

b. Place Low Calibrator in CPPC plate nest, clear side up so that plate feet seat into position. Follow prompts to count, remove and place High Calibrator to count _____

c. Low Calibrator and High Calibrator produce results in expected ranges _____

1. If insertion of a calibrator results in a placement error message, remove and re-insert _____

2. If calibrators are out of range, do not proceed**;** seek technical assistance _____

2. Sort plates by type and matrix; then select test channel PPAC or PPCC/ PPEC and appropriate matrix _____

a. An administrator may create a new channel or matrix; refer to manufacturer’s instructions _____

b. Rehydrate a fresh Peel Plate with appropriately diluted matrix (this plate is not to be incubated) and use as a background for new channel setup (refer to CPPC equipment manual). Press Background button on Admin tab and accept as the background image. _____

3. Examine each test plate visually prior to placing into the CPPC _____

a. For atypical plates; spreader colonies, confluent growth, excessive growth around edge of plate, etc., do not count with CPPC, record as appropriate using items 15 & 16 _____

4. Place Peel Plate in platform, adhesive film down and clear side up, seating feet into the CPPC plate nest _____

5. Enter Sample ID and press Count/Accept _____

6. Review the count/image _____

   a. If count does not appear to agree with visual inspection, click on image to review counted colonies and to allow for a manual adjusted count _____

      1. The CPPC count may be corrected by overwriting the count with the visual count. In the automatically recorded result, M precedes the manual count and the CPPC count appears in parenthesis _____

      2. Dilution factor and Peel Plate lots and expiration may also be changed on the edit table _____

   b. Manual count prompt to count plate will automatically appear if large colonies, spreaders or TNTC counts are detected. Press OK and edit table appears for corrections, item 14.c.6. a. _____

   c. Record count result by placing the next plate to be counted into plate nest and pressing Accept Count/Next button _____

7. Repeat steps 14.c.2-4, or 14.c.3-4 if same test and matrix. Previous count and manual edits are accepted, recorded and placed in memory _____

8. Results and images may be downloaded as .csv. and .pdf files. Results may also be printed. Refer to manufacturer's instructions _____

9. Maintain records _____

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 see item 18.a.3. _____

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/PPCC/PPCCD/ PPECCD and PPECHV/PPCCHV/PPCCCDHV/PPECCDHV _____

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 24±2, or 48±3 hours if using bisulfite diluent, 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 and 4.a.3), 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 _____

b. If using an approved Charm Peel Plate Counter (CPPC, item 14.c) analysts must perform monthly visual counts comparing to CPPC results (CPPC = one analyst) using a plate in the countable range _____

1. If only one analyst, count must be ≤ 10% between visual and the CPPC result; maintain records _____

2. With two or more analysts, use the RpSm method (see current SMEDP); using the CPPC result as an analyst count; maintain records _____

REPORTING

18. Reporting _____

[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 exceed 30 per cm.sq., compute count by multiplying count in representative 1 sq.cm, or average count in 5 representative squares, x dilution factor x sq. area of plate (1 mL plate=17.4 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, PPCC/PPEC/PPCCCD/PPECCD _____

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

a. For chocolate milk 1:2 dilutions plated in duplicate, sum results and report as coliform/mL (PPCC/PPEC/PPCCCD/PPECCD/mL) _____

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

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

c. High Sensitivity Total Coliform, PPCCHV/PPECHV/PPCCCDHV/PPECCDHV _____

1. Report count for 1:5 dilution in a single plate or 1:10 dilution in duplicate plates, sum results and report as coliform/mL or g (PPCCHV/PPECHV/ PPCCCDHV/PPECCDHV) _____

2. If for any reason an entire plate is not counted, the computed count is reported as Estimated (EPPCCHV/EPPECHV/EPPCCCDHV/EPPECCDHV) _____

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) _____