PETRIFILM® AEROBIC, PETRIFILM® RAPID AEROBIC, AND PETRIFILM® COLIFORM METHODS
IMS #5a (PAC), IMS #5b (RAC), IMS #20a (PCC, HSCC)

[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. Petrifilm Aerobic Count (PAC), Petrifilm Rapid Aerobic Count (RAC), Petrifilm Coliform Count (PCC) & Petrifilm High Sensitivity Coliform Count (HSCC) Plates _____

3. Plastic Spreaders (Manufacturer supplied) _____

a. PAC – concave, ridge side used _____

b. RAC - flat spreader _____

c. PCC – smooth, flat side used _____

d. HSCC – large spreader _____

PROCEDURE

4. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

5. Selecting Dilutions _____

a. PAC/RAC _____

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. Not performed on cultured or acidified products _____

b. PCC _____

1. For pasteurized fluid milk samples, 1 mL direct and/or decimal dilutions, as appropriate (see item 5.c.2 below) _____

2. For samples other than milk (item 12) distribute 10 mL of a 1:10 dilution among ten (10) PCC plates, 1 mL per plate or use HSCC plates (see 5.c below) _____

c. HSCC _____

1. At least a 1:5 minimum dilution required for: cottage cheese, evaporated milk, heavy and light cream, sweetened condensed milk and eggnog (flavored milk optional) _____

2. A 1:10 minimum dilution required for: sour cream, yogurt, and sour cream based dips (flavored milk optional) _____

3. Test 5 mL of 1:5 dilution (5 mL on 1 plate) or test 10 mL of 1:10 dilution (5 mL on 2 plates); generally high fat and viscous products _____

d. For acidified products, add 1.0 N NaOH drop wise (approx. 0.1 mL per gram of product) to sample dilution blank until small portion tested (pH paper or pH meter/probe) falls within the following: _____

1. PCC – pH range 6.6 to 7.2 _____

2. HSCC – 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 _____

6. Identifying Petrifilm Plates _____

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

7. Controls (AM and PM) _____

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

b. Expose a rehydrated 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 plate with 1 mL dilution buffer as described in items 10.h or 11.i _____

b. Drop the top film down onto dilution buffer and spread as described in items 10.h.2 & 10.i or 11.i.2 & 11.j _____

c. Leave plate undisturbed for 1-2 min _____

d. Roll top film back and completely expose both rehydrated surfaces for 15 min; timer used _____

e. After 15 min, roll top film back down and incubate as described in item 14 _____

2. After incubation, PAC air plate(s) shall contain ≤ 10 colonies. RAC air plate(s) shall contain ≤15 colonies _____

3. Take and record corrective actions for air control plate(s) over these defined limits _____

c. Maintain records _____

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

DILUTING SAMPLES

8. Sample Agitation _____

a. When appropriate, wipe top of unopened containers with 70% 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 _____

9. 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 (see CP GR item 22)

PLATING

10. Sample & Dilution Measurements, pipets

a. Use separate sterile pipets for the initial transfers from each container, adjusting 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. Lift the top film and deposit 1 mL (PAC/RAC/PCC), or 5 mL (HSCC) of sample or dilution keeping pipet nearly vertical

1. Release sample or dilution portion onto the center (PAC/RAC) or just above the center (PCC & HSCC) of the plate base film with tip slightly above but not in contact with plate base film with a 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. PAC/RAC – Carefully **drop** the top film onto the inoculum _____

3. PCC – Carefully **roll** the top film onto the inoculum to prevent trapping bubbles _____

4. HSCC – Carefully **roll** the top film onto the inoculum gently to prevent pushing the inoculum off the bottom film and to avoid trapping air bubbles _____

i. Place the appropriate plastic spreader (item 3) on the top film over the inoculums _____

1. PAC – gently press down on the center of the spreader (ridge side down) to distribute inoculum to the circular ridge of the spreader _____

2. RAC – gently press down on the center of the spreader to distribute inoculum over the growth area _____

3. PCC – gently press down on the center of the spreader (flat side down) to distribute inoculum over the growth area _____

4. HSCC – distribute inoculum with a gentle downward pressure on the handle of the spreader until the inoculum reaches the circular ridge of the spreader _____

j. Leave plates undisturbed for gel solidification: _____

1. 1 min for PAC, RAC & PCC _____

2. 2-5 min for HSCC _____

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) _____

11. 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 cm below sample or dilution surface (avoid foam and bubbles) _____

g. With 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 film and deposit 1 mL (PAC/RAC/PCC), or 5 mL (HSCC) of sample or dilution keeping pipettor nearly vertical _____

1. Release sample or dilution portion onto the center (PAC/RAC) or just above the center (PCC & HSCC) 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. PAC/RAC – Carefully drop the top film onto the inoculum _____

3. PCC – Carefully roll the top film onto the inoculum to prevent trapping bubbles _____

4. HSCC – Carefully roll the top film onto the inoculum gently to prevent pushing the inoculum off the bottom film and to avoid trapping air bubbles _____

j. Place the appropriate plastic spreader (item 3) on the top film over the inoculums _____

1. PAC – gently press down on the center of the spreader (ridge side down) to distribute inoculum to the circular ridge of the spreader _____

2. RAC – gently press down on the center of the spreader to distribute inoculum over the growth area

3. PCC – gently press down on the center of the spreader (flat side down) to distribute inoculum over the growth area

4. HSCC – distribute inoculum with a gentle downward pressure on the handle of the spreader until the inoculum reaches the circular ridge of the spreader

k. Leave plate undisturbed for gel solidification

1. 1 min for PAC, RAC & PCC

2. 2-5 min for HSCC

l. 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)

12. Samples Other than Milk

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

13. Dry Dairy 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

14. Incubating Petrifilm Plates (see CP item 15)

a. Stack plates in horizontal position, clear side up

1. PAC/RAC/PCC - no more than 20 high

2. HSCC - no more than 10 high

b. Incubate within 10 min

1. PAC - 48±3 hours at 32±1°C

2. RAC - 24±2 hours at 32±1°C (all dairy products except powders)

3. RAC - 48±3 hours at 32±1°C (all dairy powders)

4. PCC/HSCC - 24±2 hours at 32±1°C

COUNTING COLONIES

15. Counting Aids (see CP item 17) _____

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 an approved Petrifilm reader _____

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

2. Petrifilm Plate Reader (PPR) [Approved for use with PAC only] _____

a. Store System Verification Cards (SVC) in a clean, dry and enclosed container _____

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

1. Use Log File feature to automatically save electronic pass/fail result _____

c. Scan and record SVC result hourly with continuous operation _____

1. Use Log File feature to automatically save electronic pass/fail result _____

d. SVC used to check the function of the PPR prior to reading test PAC plates _____

1. Exp. Date:_____ _____

2. If inserting the SVC results in an error message, remove and re-insert card _____

3. If an error occurs a second time, inspect card for visible dirt or defects, clean and re-insert card _____

4. If card gives a third error, replace card. Scan and report results of new card _____

5. Do not proceed unless SVC gives an acceptable result; seek technical assistance _____

3. Petrifilm Plate Reader Advanced (PPRA) [Approved for use with PAC only] _____

a. Run the Petrifilm Plate Manager Software Imaging System Verification to confirm the PPRA imaging system is working properly on start-up _____

b. If the system fails, follow the instructions on the display screen. If the reader does not give the appropriate prompt, reach out to the manufacturer.

4. Advanced Instruments® PetriScan® Reader
[Approved for use with PAC only]

a. Inspect scanner glass for spots and if necessary clean using a soft, lint-free cloth with a mild glass cleaner

b. Place templates 1 and 2, and two PAC plates with no growth in the PetriScan grid and scan

c. Count and record all results prior to the start of and at the end of each operation period

d. Scan, count and record template and no growth PAC plate results hourly with continuous operation

e. Template 1 gives count between 27 and 33

f. Template 2 gives count between 190 and 210

g. No growth PAC plates give a count of zero

h. If any results out of range

1. Inspect templates and PAC plates for defects and scanner glass for spots

2. If defect(s) found, replace template or PAC plates and scan, count and record new result(s)

3. Do not proceed until template and no growth PAC plates give acceptable results, seek technical assistance

6. Maintain records

d. Examine each test plate visually prior to placing into the reader

1. For plates with no growth, assure reader count is Zero

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

16. Counting, Recording and Computing PAC/RAC

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 colonies regardless of size, color, or intensity _____

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

    2. Examine edge of plate for colonies _____

    3. Count all colonies including those outside the circular indentation left by the spreader _____

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 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 (as per Neogen® manufacturer instructions) _____

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 x 1) + (0.1 x 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

17. Counting, Recording and Computing PCC and HSCC

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. Confirmed coliform colonies are red colonies having 1 or more gas bubbles within 1 colony diameter, (No further confirmation is required)

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, sum counts of the plates

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

18. Identifying Counting Errors

a. Perform monthly counting for PAC/RAC

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 Petrifilm Plate reader (item 15.c) analysts must perform monthly visual counts comparing to reader results (reader = one analyst)

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

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

REPORTING

19. Reporting (see CP item 35.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. PAC

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

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

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

4. If computed counts from PAC plates >250, report as Estimated PAC (EPAC)

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

b. RAC

1. Report computed count as Petrifilm Rapid Aerobic Count/mL or /g (RAC/mL or RAC/g) when taken from plate(s) in the 25-250 range

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

3. When colonies on RAC plates exceed 100/sq. cm, compute count by multiplying 100 x dilution factor x 30 sq. cm and report as > computed count Estimated (ERAC)

4. If computed counts from RAC plates >250, report as Estimated RAC (ERAC)

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

c. PCC and HSCC

1. Report count as Petrifilm Coliform Count/mL or /g (PCC/mL or PCC/g) when taken from plate(s) in the 1-154 range

2. If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as Estimated (EPCC)
3. Counts from coliform plates > 154 are reported as > 150 Estimated Petrifilm Coliform Count (EPCC)

4. 5 mL of a 1:5 dilution provides a 1:1 sensitivity (HSCC)

5. 5 mL of a 1:10 dilution provides a sensitivity of 2 coliform/mL or g, run 1:10 dilutions in duplicate to get a sensitivity of 1 coliform/mL or g as required by the PMO (HSCC)

6. If for any reason, an entire plate is not counted, the computed count is reported as Estimated (EPCC or EHSCC)

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, 235 to 240)

b. When the second digit is even round down (even down, 225 to 220)

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)