PLATE LOOP COUNT
PETRIFILM™ AEROBIC AND PETRIFILM™ RAPID AEROBIC COUNT METHODS
IMS #3b (PPLC), IMS #3c (PRPLC)

[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] _____

PRE-REQUISITE

2. Comparative Test with Petrifilm Aerobic Count (PAC) or Petrifilm Rapid Aerobic Count (RAC) (2400a-4) _____

a. Analysts certified for PAC/RAC _____

b. Comparisons done by each analyst performing test _____

1. Comparison is valid only if done using similar plate count methods, i.e. Petrifilm with pipets (or pipettors) to Petrifilm with the PLC device _____

2. Results must be evaluated by a LEO and shown to be acceptable prior to official use of test in laboratory _____

3. Copy of comparison and results in QC record (or easily accessible on file in laboratory); kept for as long as analyst is certified _____

MATERIALS AND APPARATUS

3. 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 No. 54 but not a No. 53 twist drill bit (lab must have a set), checked monthly; maintain records _____

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 _____

4. 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 _____

5. Cornwall™ Continuous Pipetting Outfit (or equivalent) _____

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 graduated cylinder each day of use; maintain records _____

c. With Luer-Lok of needle attached to Luer-Lok fitting of syringe _____

6. Petrifilm Aerobic Count (PAC) or Petrifilm Rapid Aerobic Count (RAC) Plates _____

7. Plastic Spreaders (Manufacturer supplied) _____

a. PAC - concave (ridge) side used _____

b. RAC - flat spreader _____

PREPARATION

8. Heat Treatment of Pipetting Equipment _____

a. Sterilize assembled Cornwall Continuous Pipetting Outfit (or equivalent) by wrapping in Kraft paper or in a protective autoclavable container that permits the passage of steam and autoclave at 120±1°C for 15 min _____

b. The pipetting outfit cannot be used for more than a 24-hour period of time without re-sterilization _____

9. Assembly of Complete Apparatus for Use _____

a. Carefully place end of rubber supply tube (attached to syringe) in sterile dilution buffer blank and depress syringe plunger several times to pump buffer in syringe. Assure there are no air bubbles in the 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 onto instrument control plate (performed before testing of first sample) _____

PROCEDURE

10. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

11. Identifying Petrifilm Plates _____

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

b. Label each plate with sample or control identification _____

c. Arrange plates in order before testing begins _____

CONTROLS

12. Controls (AM and PM) _____

a. Check sterility of dilution blanks and Petrifilm used for each group of samples _____

b. Expose a rehydrated PAC/RAC plate (both rehydrated surfaces completely exposed) to air during plating for 15 min; timer used _____

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 (not off to the side) _____

a. Inoculate the center of the PAC/RAC plate with 1 mL dilution buffer as described in item 14.b _____

b. Drop the top film down onto dilution buffer and spread as described in items 14.d & e _____

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 15 _____

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

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

c. Instrument control, item 9.c _____

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

e. After all samples in a series have been tested, discharge a final rinse to a control plate _____

f. Between sample series on the same day, properly protect the loop from contamination; repeat 9.b & c at the start of the next series _____

g. Maintain records _____

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

DILUTING SAMPLES

13. Sample Agitation _____

a. When appropriate, wipe top of unopened containers with 70% ethyl alcohol-saturated cloth _____

b. Before removal of any portion, mix raw milk sample(s) by shaking 25 times in 7 sec with a 1 ft movement (containers approx. ¾ full); use within 3 min _____

14. Inoculating Petrifilm Plates _____

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

b. Lift top film, position loop above center of the base film and depress plunger causing sterile dilution buffer to flow down the shank, across charged loop washing measured 0.001 mL of sample onto plate _____

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

d. Carefully drop the top film onto the inoculum _____

e. Place the appropriate plastic spreader on the top of the plate over the inoculum. _____

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 _____

f. Leave plate undisturbed 1 min _____

INCUBATION

15. Incubating Petrifilm Plates (see CP item 15) _____

a. Stack plates in horizontal position, clear side up, no more than 20 high and incubate within 10 min of gel solidification _____

b. Incubate Petrifilm Plate Loop Count (PPLC) plates at 32±1°C for 48±3 hours _____

c. Incubate Petrifilm Rapid Aerobic Plate Loop Count (PRPLC) plates at 32±1°C for 24±2 hours _____

COUNTING COLONIES

16. 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 Plate 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 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 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 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 plates give a count of zero
   h. If any results out of range
      1. Inspect templates and plates for defects and scanner glass for spots
      2. If defect(s) found, replace template or plates and scan, count and record new result(s)
      3. Do not proceed until template and no growth plates give acceptable results, seek technical assistance
5. 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 item 17

17. Counting, Recording and Computing PPLC/PRPLC

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 number of colonies on the 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. 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 _____

g. If plate exceed 250 colonies, estimate (as per manufacturer instructions) _____

h. If plate yields < 25 colonies, record actual number _____

i. If plate shows no colonies, record count as 0 _____

j. Multiply number of colonies (or estimated number if necessary) by 1,000 _____

18. Identifying Counting Errors _____

a. Perform monthly counting for PPLC/PRPLC _____

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

2. With two analysts, comparative counts agree within ≤10%, of one another; 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 16.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. PPLC _____

1. Report computed count as Petrifilm Plate Loop Count/mL (PPLC/mL) when taken from a plate in the 25-250 range _____

2. Report PPLC plate counts of 0 to 24 as < 25,000 Estimated Petrifilm Plate Loop Count/mL (EPPLC) _____

3. When colonies on plate exceeds 100/sq. cm, compute count by multiplying 100 x dilution factor x 20 sq. cm and report as > computed count Estimated (EPPLC) _____

4. If computed counts from PPLC plate is > 250, report as Estimated PPLC (EPPLC) _____

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

b. PRPLC _____

1. Report computed count as Petrifilm Rapid Plate Loop Count/mL (PRPLC/mL) when taken from a plate in the 25-250 range _____

2. Report PRPLC plate counts of 0 to 24 as < 25,000 Estimated Petrifilm Rapid Plate Loop Count/mL (EPRPLC) _____

3. When colonies on plate exceeds 100/sq. cm, compute count by multiplying 100 x dilution factor x 20 sq. cm and report as > computed count Estimated (EPRPLC) _____

4. If computed counts from PRPLC plate is > 250, report as Estimated PRPLC (EPRPLC) _____

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

c. Report only first two left-hand digits _____

1. If the third digit is 5 round the second number using the following rules _____

2. When the second digit is odd round up (odd up, 235 to 240) _____

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

e. If the plate from a sample has 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) _____