PLATE LOOP COUNT
3M™ PETRIFILM™ AEROBIC COUNT METHOD
IMS #3

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

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

PRE-REQUISITE

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

a. Analysts certified for PAC   _____

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) Plates _____

7. Plastic Spreader _____

    a. Provided with PAC plates, concave (ridge) side used _____

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 into 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 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 plating (not off to the side) _____

            a.   Inoculate the center of the PAC 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, air plate(s) shall contain ≤ 10 colonies _____

        3.   Take and record corrective actions for air control plate(s) with
> 10 colonies _____

    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 sterile, 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 plastic spreader on the top of the plate over the inoculum. Gently press down on the center of the spreader (ridge side down) 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 PPLC plates at 32±1°C for 48±3 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.   3M Petrifilm Information Management System (PIMS)   _____

             a.   Store control cards in a clean, dry and enclosed container   _____

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

             c.   Scan and record control card result hourly with continuous operation   _____

             d.   Control card result must fall in the 92 to 108 range, if outside of this range an alarm will sound to alert the operator of a failure   _____

                 1.   Exp. Date: _____   _____

                 2.   If alarm sounds, inspect card for defects, if defect(s) are observed replace control card, scan and report result of new card   _____

                 3.   Do not proceed unless control card gives acceptable result, seek technical assistance   _____

         3.   3M Petrifilm Plate Reader (PPR)   _____

             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 result of new card _____

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

4. Advanced® Instruments PetriScan® Reader _____

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 plate 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 _____

a. After incubation count all colonies on 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 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, even those outside the circular indentation left by the spreader _____

f. Spreaders 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 exceeds 250 colonies, estimate (as per 3M 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   _____

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

2.   With two analysts, comparative counts agree within $\leq$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 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.   Report computed count as Petrifilm Plate Loop Count/mL (PPLC/mL) when taken from a plate in the 25-250 range   _____

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

c.   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)   _____

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

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

f. 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)   _____

g. If the plate from a sample has excessive spreader colony growth or liquefiers, report as spreaders (SPR) or liquefiers (LIQ)   _____

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