PLATE LOOP COUNT
AGAR POUR PLATE METHOD
IMS #3a

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

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

PRE-REQUISITE

2. **Comparative Test with Standard Plate Count (SPC) (SPC – Agar Pour**
    Plate Form 2400a)   _____

    a.   Analyst(s) certified for SPC   _____

    b.   Comparisons done by each analyst performing test   _____

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

         2.   Results must be evaluated by a State/Federal 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 _____

PREPARATION

6. Media Preparation (reference agar from CP items 14, 27, 28 & 29) _____

a. Temperature control (TC) used _____

1. Contains agar identical to type and volume being used _____

2. In container identical in size and volume to that being used _____

3. Undergoes same heat treatment and cooling as test agar _____

b. Plate Count Agar or Standard Methods Agar (PCA or SMA) _____

1. Prepare and sterilize agar for sample series and all controls _____

2. OR use previously prepared/stored agar; melt agar quickly in boiling water or flowing steam; not under pressure _____

3. Do not melt agar more than once _____

4. Promptly place in a circulating water bath to temper and hold melted agar at 45±1°C _____

5. Record agar temperature with other control information _____

6. Discard agar that is not used within 3 hours after tempering _____

7. Avoid prolonged exposure to high temperatures during and after melting; establish lab protocol _____

7. 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 _____

8. 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 that 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 into instrument control plate (performed before testing of first sample) _____

PROCEDURE

9. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

10. Identifying Plates _____

a. Select number of samples in any series so that all will be plated within 20 min (pref. $\leq$10 min) after depositing the first sample and pouring the last plate in the series _____

b. Label each plate with sample or control identification _____

c. Arrange plates in order before testing begins _____

CONTROLS

11. Controls (AM and PM) _____

a. Check sterility of dilution blanks, agar, and Petri dishes used for each group of samples _____

b. Expose a poured plate to air with cover completely removed during plating for 15 min; timer used _____

1. The air control plate must be the first plate poured 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) _____

2. After incubation, air plate(s) shall contain $\leq$15 colonies _____

      3.   Take and record corrective actions for air control plate(s)
with >15 colonies   _____

c.   Instrument control, see item 8.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 8.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

12. 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   _____

13. Inoculating 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.   Raise cover of Petri dish (just high enough to insert loop), insert loop
and depress plunger causing sterile dilution buffer to flow down the
shank, across charged loop washing measured 0.001 mL of sample
into dish   _____

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

14. Pouring Agar   _____

a.   After dispensing test portions, promptly pour 10-12 mL agar into
each plate of series, or 12-15 mL where agar weight loss is a problem
that cannot be corrected by other actions (Documentation must be kept
to indicate that this is a routine practice; amount poured to match agar
weight loss test)   _____

1. Lift cover of Petri dish just high enough to pour agar   _____

2. After agar is poured, thoroughly and evenly mix agar and test portion in Petri dish(es)   _____

   a. Agar solidification to occur within 10 min   _____

   b. Do not stack plates prior to solidification   _____

INCUBATION

15. Incubating Plates (see CP item 15)   _____

a. Stack plates (upside down) no more than 6 high and incubate within 10 min of agar solidification   _____

b. Place stacks to ensure adequate air flow   _____

c. Incubate PLC plates at 32±1°C for 48±3 hours   _____

COUNTING COLONIES

16. Counting Aids (see CP items 16 & 17)   _____

a. Count colonies with Quebec dark-field model or equivalent with satisfactory grid plate (see CP item 16)   _____

b. Hand tally (see CP item 17)   _____

17. Counting, Recording and Computing PLC   _____

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 hr (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 free colony plates with 25-250 colonies and count all colonies including those of pinpoint size   _____

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

   2. Examine edge of Petri dishes for colonies   _____

    f.   Spreaders   _____

        1.   Count colonies on representative portion only when colonies are well distributed and area covered or repressed does not exceed 25% of plate   _____

        2.   Do not count if repressed growth area > 25% of plate area   _____

        3.   When spreaders must be counted, count each as a single colony   _____

        4.   Count chains/spreaders from separate sources as separate colonies   _____

        5.   If 5% of plates are more than 25% covered by spreaders, take Immediate steps to eliminate and resolve problem   _____

    g.   If plate exceeds 250 colonies, estimate counts as follows   _____

        1.   Count colonies in portions representative of distribution and estimate the total   _____

        2.   Where there are < 10 colonies/sq. cm, count colonies in 12 squares, selecting 6 consecutive squares horizontally across the plate and 6 consecutive squares at right angles   _____

        3.   When there are 10 or more colonies/sq. cm, count 4 random representative squares   _____

        4.   Multiply average number colonies/sq. cm by area of plate in sq. cm   _____

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

    i.   If the 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 PLC   _____

        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   _____

REPORTING

19. Reporting – refer to 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 Plate Loop Count/mL (PLC/mL) when taken
       from a plate in the 25-250 range _____

   b. Report PLC plate counts of 0 to 24 as < 25,000 Estimated PLC (EPLC) _____

   c. When colonies on plate exceed 100/sq. cm, compute count by multiplying
       100 x dilution factor x area of plate in sq. cm and report as > computed
       Count Estimated (EPLC) _____

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

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

   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 growth, report as
       spreaders (SPR) _____

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