

**3M™ PETRIFILM™ AEROBIC AND COLIFORM METHODS  
IMS #5 (PAC), IMS #20 (PCC, HSCC)**

[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]** \_\_\_\_\_

**MATERIALS AND APPARATUS**

2. **Petrifilm Aerobic Count (PAC), Petrifilm Coliform Count (PCC) & Petrifilm High Sensitivity Coliform Count (HSCC) Plates** \_\_\_\_\_
3. **Plastic Spreaders (Manufacturer supplied)** \_\_\_\_\_
- a. PAC – concave (ridge) side used \_\_\_\_\_
  - b. PCC – smooth, flat side used \_\_\_\_\_
  - c. 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 \_\_\_\_\_
  - 1. Plate two decimal dilutions per sample \_\_\_\_\_
  - 2. Select dilutions that would be expected to yield one plate with 22-250 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. PAC 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)

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c. HSCC

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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

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- 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:

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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

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## 6. Identifying Petrifilm Plates

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- a. Select number of samples in any series so that all will be plated within 20 min (pref.  $\leq 10$ ) after diluting first sample
- b. Label each plate with sample or control identification and dilution
- c. Arrange plates in order before preparation of dilutions

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## CONTROLS

### 7. Controls (AM and PM)

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- a. Check sterility of dilution blanks, PAC plates, and pipets/tips used for each group of samples
- b. Expose a rehydrated PAC 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 PAC plate with 1 mL dilution buffer as described in items 10.h or 11.j

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- b. Drop the top film down onto dilution buffer and spread as described in items 10.h.2 & 10.i.1 or 11.j.2 & 11.k.1 \_\_\_\_\_
- 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, air plate(s) shall contain ≤10 colonies \_\_\_\_\_

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

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 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.  $\frac{3}{4}$  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/PCC), or 5 mL (HSCC) of sample or dilution keeping pipet nearly vertical
  - 1. Release sample or dilution portion onto the center (PAC) 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 – 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

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

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

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

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

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j. Leave plates undisturbed for gel solidification:

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1. 1 min for PAC & PCC

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2. 2-5 min for HSCC

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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)

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**11. Sample & Dilution Measurements, Pipettors [for electronic pipettors, follow manufacturer instructions]    Mechanical \_\_\_\_\_    Electronic \_\_\_\_\_**

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a. Each day before use, vigorously depress plunger 10x to redistribute lubrication and assure smooth operation (mechanical pipettors)

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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

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c. Use separate sterile tip for the initial transfers from each container

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d. Depress plunger to first stop (mechanical pipettors)

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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

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f. Insert tip approximately 0.5-1.0 cm below sample or dilution surface (avoid foam and bubbles)

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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

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- 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/PCC), or 5 mL (HSCC) of sample or dilution keeping pipettor nearly vertical \_\_\_\_\_
  - 1. Release sample or dilution portion onto the center (PAC) 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 – 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. PCC – gently press down on the center of the spreader (flat side down) to distribute inoculum over the growth area \_\_\_\_\_
  - 3. 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 & 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 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**

**14. Incubating Petrifilm Plates (see CP item 15)** \_\_\_\_\_

- a. Stack plates in horizontal position, clear side up \_\_\_\_\_
  - 1. PAC/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. 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. 3M Petrifilm Information Management System (PIMS) [Approved for use with PAC only] \_\_\_\_\_
    - 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) [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 result of new card \_\_\_\_\_
5. Do not proceed unless SVC gives an acceptable result; seek technical assistance \_\_\_\_\_

4. Advanced<sup>®</sup> Instruments PetriScan<sup>®</sup> 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 \_\_\_\_\_

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 items 15 & 16

**16. Counting, Recording and Computing PAC**

- 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, even 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 3M 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 \_\_\_\_\_

$$N = \Sigma C / [(1 \times n_1) + (0.1 \times n_2)]d$$

- 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

$$\begin{aligned}
 N &= (244 + 28) / [(1 \times 1) + (0.1 \times 1)]0.01 \\
 &= 272 / [1.1]0.01 \\
 &= 272 / 0.011 \\
 &= 24,727 [25,000 \text{ (reported)}]
 \end{aligned}$$

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
  - 1. With 3 or more analysts, use the RpSm method (see current SMEDP); maintain records
  - 2. With two analysts, comparative counts agree within  $\leq 10\%$ ; 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  $\leq 10\%$  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. 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. 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)

c. 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)
- d. If all plates from a sample have excessive spreader colony growth or liquefiers, report as spreaders (SPR) or liquefiers (LIQ)
- e. If a laboratory accident renders a plate uncountable, report as laboratory accident (LA)