Microbial and Mycotoxins Workgroup

Completed Work to Date

During the 8 months of meetings, the Workgroup was able to produce three passing motions. The first motion required the molecular techniques to detect the presence of Salmonella and Shiga toxin producing E. coli (see 2021 CSTF Report[1]). The second motion (Appendix A) presented two different methods utilizing violet red bile glucose agar to quantify bile tolerant Gram negative (BTGN) bacteria, adapting National Conference of Interstate Milk Shipments (NCIMS) protocols to best fit the testing of cannabis and cannabis related products (Appendices B, C). The third motion was the adaptations to NCIMS 2400a-4 Petrifilm™ Aerobic & Coliform Count Methods Rev. 11/17 to utilize Petrifilm™ EB plates for BTGN testing in cannabis and cannabis related products (Appendix D).

Defined Challenges, Gaps and Actions Needed

During regular meetings, the Workgroup was able to produce valuable content for use by stakeholders to continue to develop standards for quality assurance testing of cannabis and cannabis related products. The Workgroup was unable to provide a completed final suite of test methods for all microbiological and mycotoxin screening/testing. As of December 2021, items that the Microbial and Mycotoxins Workgroup addressed and recommend the ICT to further discuss are, but not limited to, the following:

1. **Complete adaptations to NCIMS 2400a Standard Plate Count and Coliform Plate Count Rev. 10-13**
   The Workgroup did not have time to adapt the NCIMS 2400a Standard Plate Count and Coliform Plate Count document (Appendix B). Adapting to meet BTGN bacteria testing would be recommended to comply with the second passed Motion.

2. **Complete adaptations to NCIMS 2400 Cultural Procedures Rev. 10/19 (5/20)**
   Contains several items essential to the utilization of NCIMS protocols/documents. This document holds several quality assurance items the Work Group recommends for the testing of BTGN bacteria (Appendix E).

3. **Standardize method for Salmonella detection**
   The Workgroup only performed a high-level assessment of methods for Salmonella. Notes and links to resources are provided in Appendix F.

4. **Standardize method for Shiga toxin producing E. coli (pathogenic E. coli), "STEC"**
   The Workgroup only performed a high-level assessment of methods for Shiga toxin producing E coli (pathogenic E. coli). Notes and links to resources are provided in Appendix F.

5. **Standardize rapid method for the mycotoxin detection/quantification**
   The Workgroup only performed a high-level assessment of methods for rapid testing mycotoxins. Notes and links to resources are provided in Appendix F.

[1] https://apps.ecology.wa.gov/publications/SummaryPages/2103003.html

Background

Recommending utilization of matrix controls for all methods

While implementation was not discussed, the Work Group discussed the need for matrix controls to assist in methods not validated for cannabis. The matrix controls could be utilized as a "proof of concept" and would demonstrate efficacy of the method over time.

Recommending a standardized processing method

The processing of cannabis and cannabis related products for microbiological and mycotoxin testing was not addressed during our meetings. As standardized methods are selected, the processing of the matrix must also be finalized to ensure proper handling and testing of the product.

Appendix A: Motion #2

October 25, 2021

MOTION #2: UTILIZATION OF VIOLET RED BILE GLUCOSE AGAR TO QUANTIFY BILE TOLERANT GRAM-NEGATIVE BACTERIA

This motion would require the use of violet red bile glucose agar (VRBGA) to quantify bile tolerant Gram-negative (BTGN) bacteria in cannabis flower and other processed or extracted cannabis flower product using the National Conference on Interstate Milk Shipments (NCIMS) checklist/protocol(s): FORM FDA/NCIMS 2400a- Standard Plate Count and Coliform Plate Count Methods Rev 10-13, or 2400a-4 Petrifilm™ Aerobic & Coliform Count Methods Rev. 11/17 adapted for cannabis. Cannabis specific adaptations to these documents will be provided in separate, future motions.

Appendix B: NCIMS 2400a Standard Plate and Coliform Count Methods

STANDARD PLATE AND COLIFORM COUNT
AGAR POUR PLATE METHODS
IMS #2 (SPC), IMS #21 (CPC)

[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]

MEDIA PREPARATION

2. Media Preparation (reference agars/broth from CP items 14, 27, 28 & 29) _____

a. Temperature Control (TC) used for each test agar type _____

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, hold melted agar at 45 ±1°C _____
5. Record agar temperature with other control information _____
6. Agar should be discarded if not used within 3 hours after tempering _____
7. Avoid prolonged exposure to high temperatures during and after melting; establish lab protocol _____

c. Violet Red Bile Agar (VRB) _____

1. Boil for at least 1 min, but no more than 2 min. Do not autoclave. _____
2. Promptly place in a circulating water bath to temper; hold melted agar at 45±1°C _____
3. Record agar temperature with other control information _____

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4. Agar should be discarded if not used within 3 hours after tempering _____

d. Brilliant Green Lactose Bile Broth (BGB) _____

1. Examine Durham/fermentation tubes for presence of air bubbles _____

2. If air bubbles cannot be removed from tubes; DO NOT USE _____

PROCEDURE

3. Work Area _____

a. Level plating bench not in direct sunlight _____

b. Sanitize immediately before start of plating _____

4. Selecting Dilutions _____

a. Standard Plate Count (SPC) _____

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. SPC not performed on cultured or acidified products _____

b. Coliform Plate Count (CPC) _____

1. For pasteurized fluid milk samples, 1 mL direct and/or decimal dilutions as appropriate _____

2. For samples other than milk (item 11) and dry milk products (item 12) distribute 10 mL of a 1:10 dilution among three plates _____

5. Identifying Plates _____

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

b. Label each plate with sample or control identification and dilution _____

c. Arrange plates in order before preparation of dilutions _____

CONTROLS

6. Controls (AM and PM) _____

a. Check sterility of dilution blanks, agar, Petri dishes, and pipets/tips 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 ≤15 colonies _____

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

c. Maintain records _____

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

DILUTING SAMPLES

7. 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 milk 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 _____

8. 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 _____

PLATING

9. 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. Gently lift cover of Petri dish just high enough to insert pipet _____

i. Hold pipet at approximately a 45° angle with tip touching dish _____

j. Release sample or dilution portion to Petri dish (with lower side of pipet in contact with plate) with column drain of 2-4 sec _____

1. Using pipet aid, blow out the last drop of undiluted sample, away from main part of sample _____

2. On diluted samples, touch pipet tip once against dry spot on dish bottom _____

a. When depositing 0.1mL, do not re-touch to dry area _____

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) _____

10. 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. Gently lift cover of Petri dish just high enough to insert tip with pipettor approximately vertical to dish _____

1. Release sample or dilution portion onto plate with tip slightly above but not in contact with the plate by slowly depressing plunger completely _____

a. For two (2) stop pipettors, depress plunger to second stop _____

b. Do not touch off pipettor tip(s) to Petri dish _____

c. Optionally, deposit samples with pipettor capable of making a 1:10 dilution in the tip _____

j. 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) _____

11. Samples Other than Milk _____

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

12. Dry Milk Product Samples _____

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

1. Use standard dilution blank _____

2. Or, 2.0 % sodium citrate blank (pH<8.0) for relatively insoluble sample (e.g. whey) _____

b. Wet sample completely with gentle inversions _____

c. Let soak a minimum of 2 min; shake 25 times in 7 sec with a 1 ft movement; use within 3 min of agitation _____

13. Pouring Agar _____

a. After dispensing test portions, promptly pour 10-12 mL of agar into each plate of series, or 12-15 mL for > 1 mL portion/plate or 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 _____

b. For dry milk product sample(s), overlay plate with 3-5 mL PCA or SMA _____

c. For coliform count, overlay plate with 3-4 mL VRB _____

INCUBATION

14. 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 SPC plates at 32±1°C for 48±3 hours (dry milk products for 72±3 hours) _____

d. Incubate Coliform plates at 32±1°C for 24±2 hours _____

COUNTING COLONIES

15. Counting Aids (see CP items 16 and 17) _____

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

b. Hand tally (**see** CP item 17) _____

16. Counting, Recording and Computing SPC _____

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 hr (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 free 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. If consecutive plates yield 25-250 colonies, count all colonies on plates from both dilutions _____

g. Spreaders _____

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

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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 _____

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 counts as follows _____

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

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

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 CPC _____

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 hr (avoid as a routine practice) _____

c. Confirmation of colonies _____

1. Pick 10% up to 10 representative colonies per plate with relative percentages of each colony type and inoculate into BGB; incubate for 48±3 hours at 32±1°C _____

2. Gas production at any time during the incubation is considered a confirmed test _____

3. Record the number of picked colonies and the number of colonies that produced gas (if necessary calculate % confirmed and multiply by total number of colonies) _____

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 the highest dilution plate, record number as >150 _____

g. When multiple plates of a dilution are used, sum counts of 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 SPC _____

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. With only one analyst, replicate counts agree within ≤8% of one another; 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. SPC _____

1. Report computed count as Standard Plate Count/mL or /g (SPC/mL or SPC/g) when taken from plate(s) in the 25-250 range _____

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

3. When colonies on SPC plates 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 (ESPC) _____

4. If computed counts from SPC plates are >250, report as Estimated SPC (ESPC) _____

b. CPC _____

1. Report count as Coliform Plate Count (confirmed)/mL or /g when taken from plate(s) in the 1-154 range (CPC/mL) _____

2. If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as Estimated (ECPC) _____

3. Counts from coliform plates > 154 are reported as > 150 Estimated Coliform Count (ECPC) _____

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

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 growth, report as spreaders (SPR) _____

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

Appendix C: NCIMS 2400a-4 Coliform Methods

3M™ PETRIFILM™ AEROBIC, 3M™ PETRIFILM™ RAPID AEROBIC, AND 3M™ PETRIFILM™ COLIFORM METHODS
IMS #5 (PAC), IMS # (RAC), 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. **3M Petrifilm Aerobic Count (PAC), 3M Petrifilm Rapid Aerobic Count (RAC), 3M Petrifilm Coliform Count (PCC) & 3M 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 _____

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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.2 or 11.j.1 & 11.j.2 _____

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) that exceed 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 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. 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 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/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 _____

3. PCC/HSCC - 24±2 hours at 32±1°C _____

COUNTING COLONIES

15. Counting Aids _____

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 results 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 results of new card _____

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

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 _____

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/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 red colonies on PAC or all colonies on RAC 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 regardless of size, color or intensity, 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 _____

$$N = \Sigma C/[(1 \times n1) + (0.1 \times n2)]d$$

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

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

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 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. 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) _____

Appendix D: Motion #3

December 7, 2021

MOTION #3: ADAPTATIONS TO NATIONAL CONFERENCE OF INTERSTATE MILK SHIPMENTS 2400A-4 FOR TESTING OF ENTEROBACTERIA

This motion would adopt the following list of adaptations to the NCIMS 2400a-4 Petrifilm™ Aerobic & Coliform Count Methods Rev. 11/17 to utilize for the testing of cannabis and cannabis related products to quantify Enterobacteria (biletolerant gram-negative bacteria):

NCIMS 2400a-4 Enterobacteria Amendments for Cannabis and Cannabis Products

1. Change all references of milk, raw milk, and other milk products to cannabis and cannabis related products
2. Remove all references of “3M Petrifilm Aerobic (PAC)”, “3M Petrifilm Rapid Aerobic (RAC)”, “3M Petrifilm Coliform (PCC)”, and “3M Petrifilm High Sensitivity Coliform Count (HSCC)” Plates/Methods; change to “3M Petrifilm Enterobacteriaceae Count (EB) Plates/Method”. All procedures and steps specific to PAC, RAC, PCC, and HSCC techniques should be removed in subsequent adaptations.
3. Include “EB plate spreader – flat side down” to Section 3
4. Change “raw milk” in Section 5.2.a to “cannabis” and change “finished products” in Section 5.2.b to “cannabis related products”; strike Section 5.b, 5.c, and 5.d
5. Include manufacturer’s product information of efficacy check into Section 7 (see Resources)
6. Strike Sections 8.b.1 and 8.b.2
7. Replace references of “foam and bubbles” (Sections 10 and 11) to “particulates”
8. Strike Sections 10.h.2, 10.h.4, and 10.j.2
9. Strike Sections 12 and 13
10. Strike Section 14.a.2
11. Incubation time and temperature would align with equivalent, standard culture methods (32C+/- 1C, 24H+/- 2J); strike Sections 14.b.2 and 14.b.3
12. Strike Section 15.c
13. Strike Section 17
14. Strike Section 18.b
15. Strike Sections 19.b and 19.c

Appendix E: Cultural Procedures General Requirements

CULTURAL PROCEDURES-GENERAL REQUIREMENTS

FDA/NCIMS Revision 10/19

[Unless otherwise stated all tolerances are ±5%]

APPARATUS & MATERIALS

1. Work Area _____

a. Level table or bench, ample working space and utilities _____

b. Clean, well ventilated, temperature 16-27°C reasonably free from dust and drafts _____

c. Well-lighted, > 50 foot-candles at working surface (pref. 100) _____

d. Microbic density of air ≤ 15 colonies/SPC or RAC plate, ≤ 10 colonies/PAC plate or ≤ 5 colonies/PPAC plate in 15 min exposure; if not, corrective actions taken (for plating procedures only) _____

e. Freedom from congestion and traffic; only compatible laboratory functions performed _____

f. Safe working environment – Refer to OSHA _____

1. Eating and drinking not permitted in laboratory _____

2. Food and drinks for consumption not stored in laboratory _____

3. Analyst wear buttoned/snapped lab coats/uniforms and protective eye-wear, lab coats/uniforms remain on-site _____

4. Safety equipment available _____

5. Current Safety Data Sheets (SDS) accessible to analysts _____

6. Has functioning fume hood with acceptable sash (if necessary, see DMSCC procedure) _____

7. Flammable solvent areas continuously well ventilated and temperature controlled _____

8. Proper disposal of potentially hazardous materials _____

a. Contaminated samples disposed of properly _____

b. Contaminated glassware or plasticware disposed of or decontaminated properly _____

c. Hazardous chemical disposed of properly _____

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g. Storage Space _____

1. Cabinets, drawers, and shelves adequate _____

h. Areas neat, clean and orderly _____

i. Floors clean, walls and ceilings in good repair _____

j. Laboratory free of insects and rodents _____

2. Records _____

a. All laboratory related records maintained and available for announced surveys _____

1. Three (3) years for state central labs _____

2. Two (2) years for other labs, minimum requirement (States may require longer periods) _____

b. Quality control and sample records available to laboratory evaluation officer during survey _____

c. Records contain written corrective actions when taken _____

d. Records written in ink or other indelible substance, pencil or erasable ink not allowed _____

e. Corrections to quality control records, bench sheets and reports follow the requirements below: _____

1. Make a single line through the incorrect information _____

2. Write in the correct information next to the incorrect information _____

3. Person making the correction initials the information _____

4. If not obvious, include reason for correction _____

f. Requirements for electronic/computer records _____

1. Software must be well documented. General software description including who is allowed to make modifications _____

2. Protocols and policies are documented clearly. Policy statement on the use of the software _____

3. Records must be indexed and cross referenced to allow easy review, or must be printed and made available. Records will allow tracking of sample from submission to final report _____

4. When corrections are necessary the old information must be retained in some form, the person making the change must be identified, the date of the change noted, and the reason for the change noted _____

5. Regulatory records archived for a period of two years (three years for State Central Labs); same as retention time for paper records _____

6. If records are not available at time of audit, facility will be cited for not having records and will be subject to penalties _____

3. Temperature Measuring Devices _____

a. National Institute of Standards and Testing (NIST) traceable temperature measuring device, or equivalent, with certificate. Check annually at ice point _____

1. Reference temperature measuring device identity: _____

	Serial #	Date of Certificate	Ice Point Date	
a:	_____	_____	_____	_____
b:	_____	_____	_____	_____
c:	_____	_____	_____	_____
d:	_____	_____	_____	_____

2. Graduation interval not more than 0.5°C (0-100°C) otherwise not more than 1.0°C (< 0 or >100°C) _____

b. Range of test temperature measuring device appropriate for designated use _____

1. Mercury-in-glass (MIG), alcohol/spirit (AIG) or electronic/digital thermometers in degrees centigrade _____

2. Plastic lamination recommended for mercury thermometers _____

3. Graduation/recording interval not more than 0.5°C (0-100°C) otherwise not more than 1.0°C (< 0 or >100°C) _____

c. Accuracy of all test temperature measuring devices, including those for autoclaves and hot air ovens checked before initial use and annually _____

1. Checked against NIST traceable thermometer _____

2. Accurate to ±1°C when checked at temperature(s) of use _____

3. Record/document results; tag individual devices _____

a. Tag includes identification/location, date of check, temperature(s) checked and correction factor(s), as applicable _____

d. Temperature measuring devices are to be read to the nearest graduation/recording interval, optionally labs may interpolate between graduations _____

e. Temperature Monitoring Systems (wired/wireless) _____

1. The software must record temperature reading from each sensor/probe in the piece of equipment being monitored at the same or greater frequency as stipulated for MIG or AIG thermometers. Optionally, set to register an alert/alarm when out of the acceptable temperature range _____

a. When temperature(s) are out of acceptable range for greater than two hours, event must be documented and corrective action taken as necessary; maintain records _____

2. Optionally, a minimum two-day backup power source (battery/electrical) for the temperature monitoring system and/or all required sensors/probes, remote signal devices and monitor/controller may be employed in case of power failure _____

3. Temperature monitoring system records for each piece of equipment must be available/accessible for auditing as described in item 2.f above _____

f. Automatic temperature recording instruments, other than those described in section 3.e that meet the requirements of 3.c., if used, compared weekly against an accurate thermometer; record results _____

g. Dial thermometers not used in the laboratory _____

4. Refrigeration (Sample _____) _____

(Reagent _____) _____

a. Size adequate for workload _____

b. Maintains samples at 0.0-4.5°C; if temperature out of range, record samples as not analyzed (NA) _____

c. Used for storage of milk or milk products, media and reagents only _____

1. Not to be used to store food or drink for consumption _____

d. Record/download temperature (corrected) daily, in AM and PM, from two temperature measuring devices with bulbs or sensor/probe immersed in liquid (in sealed containers) _____

e. Temperature measuring devices located on upper and lower shelves of use _____

5. Freezer (_____) _____

a. Size adequate for workload _____

b. Maintains -15°C or below _____

c. Used for storage of frozen milk products, controls, media and reagents only _____

1. Not to be used to store food or drink for consumption _____

d. Record/download temperature (corrected) daily, in AM and PM, from temperature measuring device with bulb or sensor/probe immersed in liquid (in sealed container) _____

6. Pipets (Glass: _____ Plastic: _____ Pipettor: _____) _____

a. Appropriate capacity _____

b. Must conform to APHA specifications _____

c. Graduations distinctly marked with contrasting color _____

d. Discard those with broken tips, scratches or other defects _____

e. Pipettors, accuracy checked, fixed volume or electronic only _____

1. Pipettors etched with identification (imprinted serial numbers acceptable) and tag with date of accuracy check _____

2. Tips (sterile for plate counts) appropriate to pipettor(s) being used _____

3. Follow manufacturer's instructions unless otherwise stated regarding proper technique for use _____

4. Check accuracy with ten (10) consecutive weighings once every 6 months (using separate tip for each weighing), average of all 10 weighings must be ±5% of specified delivery volume (by weight, or if ≥ 1.0 mL may be checked by volume using Class A graduated cylinder); maintain records _____

5. Or, check accuracy with 10 consecutive readings once every 6 months using the Artel PCS® Pipette Calibration System, average of all 10 readings must be ±5% of specified delivery volume; maintain records _____

a. PCS Calibration System Validation: upon receipt, validate the instrument by following the manufacturer's protocol _____

b. PCS Pipette System Quality Control _____

1. Following manufacturer's Procedure Guide and instrument prompts, perform an instrument calibration every 30 days or just prior to use _____

2. Record results and file Calibration Certificate (printout) _____

c. Store reagent kits and Instrument Calibrator kits at room temperature _____

Lot #: _____ Exp. Date: _____ _____

d. Reagent Blanks and Sample Solutions are the same lot _____

e. PCS Pipette Calibration System Procedure; follow manufacturer's Procedure Guide and instrument prompts _____

7. Pipet Containers _____

a. Use for sterilization, storage; non-toxic _____

8. Dilution Bottles and Closures, reusable _____

a. Bottles of borosilicate glass ___ or approved plastic ___ with smooth tops _____

b. Capacity 150 mL, indelibly marked at 99±1 mL level _____

c. Closure non-toxic rubber stopper or plastic screw cap with liner _____

d. New Bakelite type plastic caps and closures treated to remove toxic residues, tested using a *Geobacillus stearothermophilus* (A.K.A. – *Bacillus stearothermophilus*) type assay _____

e. Discard bottles and caps with chips, cracks, scratches or other defects _____

9. Petri Dishes (Glass _____ or Plastic _____) _____

a. Bottom at least 80 mm I.D., and 12 mm deep for plate counts _____

b. Bottom 86.1 – 87.0 mm I.D., and 12 mm deep for BsDA _____

c. Bottom flat and free from bubbles, scratches, or other defects _____

10. Petri Dish Container _____

a. Use for sterilization, storage; non-toxic _____

11. Hot-Air Sterilizing Oven (_____) _____

a. Sufficient size to prevent crowding of interior in normal usage _____

b. Constructed to provide uniform temperature in chamber _____

c. Temperature measuring device or recorder with adequate range (to 220°C) _____

1. Bulb or sensor/probe of temperature measuring device immersed in sand _____

d. Maintain records for each sterilization cycle including date, start-up time, time sterilization temperature reached, and length of time at sterilization temperature _____

e. Temperature indicator used each load _____

f. Performance checked with full load and recorded quarterly (preferably weekly), using spore (*Bacillus atrophaeus*) strips, include positive control check; maintain results _____

1. Brand: _____ _____

2. Lot #: _____ Exp. Date: _____ _____

12. Sterilization by Dry Heat _____

a. Material in center of load heated to ≥ 170°C for ≥ 2 hours _____

b. Oven not crowded (< 75% of shelf in gravity type, 90% in forced air type) _____

13. Autoclave (Media _____) _____

(Waste _____) _____

a. Sufficient size to prevent crowding of chamber _____

b. Thermometer or temperature recorder-controller properly located to register, chamber temperature _____

c. Has pressure gauge and properly adjusted safety valve _____

d. Connected to suitable saturated steam line or steam generator _____

e. Chamber temperature checked at least quarterly (preferably more frequently, ex. weekly with sterility check) with maximum registering thermometer or electronic high temperature data logger with full load in autoclave; record results or download and print _____

f. Cycle timing checked quarterly and found to be accurate; maintain records _____

g. Maintain records for each sterilization cycle including date, start-up time, temperature and time temperature reached, length of time at temperature, time at end of run, time removed and item(s) (Waste cycle procedures exempt from the requirements for media stated in item 14. Waste cycle procedures documented; records maintained. Procedures on file including performance checks with records) _____

1. Strip recorders that provide the above information are acceptable if strips (or copies) are maintained in permanent record, include items autoclaved, time removed and initials _____

2. Circular charts must be interpreted and must have written records to verify the information stated above _____

3. Optionally, use electronic high temperature data loggers to demonstrate chamber temperature profile of autoclave run (e.g., media preparation using manual autoclave or when printout does not show temperature during sterilization cycle); if used, download and print temperature readings _____

h. Use temperature indicator for each load _____

i. Check performance with full load and record results monthly at a minimum (preferably once during each week of use), using spore (*G. stearothermophilus*) strips or suspensions, include positive control check; maintain results _____

1. Brand: _____ _____

2. Lot #: _____ Exp. Date: _____ _____

j. Perform routine maintenance and maintain records _____

14. Sterilization by Moist Heat _____

a Autoclave media at 120±1°C _____

1. Dilution buffer blanks for 15 min (30 min optional) _____

2. Media for 15 min (sugar broths as per manufacturer instructions) _____

b. Autoclave media within 1 hour of preparation _____

c. Autoclave dilution buffer on same day prepared _____

d. Loosen stoppers or caps slightly to permit passage of steam and air _____

e. All air expelled from autoclave before pressure allowed to rise _____

f. Autoclave will reach 120±1°C within 15 min (5 min pref.) of starting air-exhaust _____

g. Properly operating and calibrated temperature gauge (not a pressure gauge) relied on to insure sterilization _____

h. After sterilization, pressure gradually reduced (≥ 15 min) and media removed promptly when atmospheric pressure is reached _____

i. Total time for media in autoclave less than 1 hour _____

15. Incubator and/or Incubator Room _____

(#1: _____) _____

(#2: _____) _____

a. Sufficient size to prevent crowding of interior _____

b. Place shelves to assure uniform temperature _____

c. Record/download corrected temperature daily, in AM and PM, from two temperature measuring devices with bulbs or sensor/probe immersed in liquid (in sealed containers) _____

d. Place temperature measuring devices on upper and lower shelves of use _____

e. Agar (10-12 mL) in SPC plates and/or (1 mL) in PAC plates or (1 mL) in PPAC plates must not lose more than 15% weight after 48 hours incubation. RAC plates must not lose more than 15% weight after 24 hours incubation _____

1. Perform agar weight loss of SPC, PAC, RAC, or PPAC plates quarterly and record results _____

a. Test minimum of two (2) plates/films per shelf in use, one on each side of shelf, preferably test 10 plates evenly distributed throughout the incubator _____

2. Take corrective action taken when criteria not met and maintain records of corrective actions _____

a. If weight loss is out of compliance take corrective actions (humidify incubator, reduce air flow, etc.) and retest as above and record _____

b. Use more agar; to use this option, laboratory must document that this amount of agar is routinely used for plating _____

16. Colony Counter _____

a. Quebec dark-field model or equivalent with satisfactory grid plate _____

17. Hand Tally, accurate _____

18. pH Meter **(Milk Lab _____)** _____

(Media Prep _____) _____

a. Electronic only, readable to 0.1 pH units _____

b. Daily calibration and slope records and maintenance log maintained when in use _____

c. Record date electrodes (double junction reference pref.) put into service (write in QC record and tag probe) Date: _____ _____

19. pH Measurement _____

a. Make all measurements at room temperature _____

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b. Standardize instrument with known buffer solutions _____

1. Use three commercially prepared standard solutions _____
2. Use each aliquot once and discard _____
3. pH 4, 7 and 10 suggested for linearity and proper function of meter _____
4. Determine slope (95-102%) _____ each time meter calibrated; maintain records _____

c. Record medium pH each time measured _____

d. Determine final (after sterilization) pH of each batch of medium before use; maintain records _____

1. Standard Methods Agar, pH 7.0±0.2 _____
2. Violet Red Bile Agar, pH 7.4±0.2 _____
3. Brilliant Green Bile Broth, pH 7.2±0.2 _____
4. PM Indicator Agar, pH 7.8±0.2 _____
5. Buffered Rinse Solution, 7.2±0.2 _____
6. Nutrient Broth, pH 6.8±0.2 _____
7. Letheen Broth, pH 7.0±0.2 _____
8. Lauryl Sulfate Tryptose Broth (LST), pH 6.8±0.2 _____
9. M-Endo Agar or Broth, pH 7.2±0.2 _____
10. Stock Phosphate Buffer, pH 7.2±0.2 _____
11. Dilution Buffer, pH 7.2±0.2 _____
12. EC-MUG, pH 6.9±0.2 _____

20. Balance _____

a. Electronic only, sensitive to ≤ 0.1 g for general laboratory purposes and proper sensitivity for accuracy checks and antibiotics _____

b. Class S or S1, or equivalent ASTM 1, 2, or 3, weights _____

1. Certificate or other verification of authenticity _____
2. Free from excessive wear, filth and corrosion _____
3. Weights within class tolerance _____

c. Check monthly with weights corresponding to normal use of balance; maintain records _____

d. Check at least annually, or when weights out of tolerance, by a qualified representative for good working order with proof of check in laboratory _____

1. Milk: _____ Date of Last Check: _____ _____

2. Media: _____ Date of Last Check: _____ _____

3. Analytical: _____ Date of Last Check: _____ _____

21. Water Baths _____

a. Thermostatically controlled to appropriate temperature(s) _____

b. Water circulation capability, baths up to 64°C _____

c. Appropriate size for work loads _____

d. Maintain suitable water level _____

22. Mechanical Dilution Bottle Shaker [If approved for use in this program] _____

23. Microwave Oven [Not for melting media] _____

24. Microbiologically Suitable (MS) Water _____

a. Type: _____ _____

b. System used: _____ _____

c. Monthly testing criteria _____

1. Standard Plate Count, Petrifilm™ Aerobic Count, Petrifilm™ Rapid Aerobic Count, or Peel Plate Aerobic Count < 1,000 colonies/mL (< 10,000 colonies/mL if stored) _____

2. Total chlorine residual negative, record as less than the detection limit of test used (ex., < 0.1 mg/L) _____

3. Resistivity exceeds 0.5 megohm/cm or conductivity is less than 2.0 µmhos/cm (µS/cm) at 25°C _____

a. Brand: _____ Std.: _____ _____

b. Test performed in another lab: _____ _____

d. Tested annually for total metals (Pb, Cd, Cr, Cu, Ni and Zn), not to exceed 0.5 mg/L for each metal and not to exceed 0.1 mg/L total for all metals _____

e. If criteria not met, take corrective action(s) and record in QC record _____

f. Maintain records _____

25. De-Ionized (DI) Water – Commercially prepared or lab prepared _____

26. Dilution Buffer and Blanks _____

a. Stock phosphate buffer (Prep. Date: _____) _____

1. Prepare in laboratory (34 g KH_2PO_4/L) with MS water; OR _____

2 Purchase commercially prepared (_____) _____

a. Lot #: _____ Exp. Date: _____ _____

3. Place in small containers (≤ 100 mL), autoclave and store in refrigerator _____

b. Stock $MgCl_2$ Solution, Optional (Prep. Date: _____) _____

1. Prepare in laboratory (38 g $MgCl_2$/L or 81.1 g $MgCl_2 \cdot 6H_20$/L) with MS water; OR _____

2. Purchase commercially prepared (_____) _____

a. Lot #: _____ Exp. Date: _____ _____

3. Place in small containers (≤ 100 mL), autoclave and store in refrigerator _____

c. Prepare dilution buffer with 1.25 mL stock buffer/L of MS water _____

1. Optionally, add 5 mL of stock $MgCl_2$/L of MS water _____

d. Fill dilution bottles to contain 99±2 mL dilution buffer after sterilization _____

1. After sterilization and after cool visually observe and discard any blanks with < 97 or > 101 mL _____

2. Of remaining blanks appearing to have the correct volume, check 1 blank for every 25 that were made using a Class A graduated cylinder (or equivalent) _____

3. Maintain records of volume checks, including batch size _____

4. If any blanks out of tolerance, discard entire lot; record lot as discarded _____

e. Test blanks at 6 month intervals for toxic substances _____

1. Plate milk dilution at 0, 15, 30, 45 min _____

2. If the 45 min count is 20% less than 0 min count, determine cause and retest after correction made; maintain records _____

f. Alternatively, use commercially prepared dilution buffer blanks _____

Brand: _____ _____

Lot #: _____ Exp. Date: _____ _____

1. Maintain volume records as above _____

2. Check toxicity as above on each new lot received _____

3. Check pH and record _____

g. Maintain records _____

h. Take corrective action when criteria not met; maintain records _____

27. Reagent Chemicals – of ACS Grade _____

28. Media [Follow manufacturer's instructions unless otherwise stated] _____

a. Use dehydrated medium of correct composition _____

1. Store as specified by manufacturer; after opening, each bottle tightly capped following each use _____

2. Commercially sealed medium kept no longer than manufacturer's expiration date _____

3. Opened bottles used until manufacturer's expiration date _____

4. Discard if any change is noted in appearance or hydration regardless of manufacturer's expiration date _____

b. Plate Count Agar (PCA): _____ _____

1. Composition:

Pancreatic Digest of Casein……..	5 g	_____
Yeast Extract……	2.5 g	_____
Glucose……..	1 g	_____
Agar……	15 g	_____
MS water to make……	1 L	_____

2. Lot #: _____ Exp. Date: _____ _____

c. 3M™ Petrifilm™ Aerobic Count (PAC) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

d. 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

e. Charm® Peel Plate® Aerobic Count (PPAC) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

f. Violet Red Bile Agar (VRBA): _____ _____

1. Composition:

Yeast Extract	3 g	_____
Peptone or Gelysate	7 g	_____
Bile Salts	1.5 g	_____
Lactose	10 g	_____
Sodium Chloride	5 g	_____
Neutral Red	0.03 g	_____
Crystal Violet	0.002 g	_____
Agar	15 g	_____
MS water to make	1 L	_____

2. Boil 2 min, temper and use within 3 hours (do not autoclave) _____

3. Lot #: _____ Exp. Date: _____ _____

g. 3M™ Petrifilm™ Coliform Count (PCC) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

h. 3M™ Petrifilm™ High Sensitivity Coliform Count (HSCC) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

i. Charm® Peel Plate® Coliform Count (PPCC or PPCCCD) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

j. Charm® Peel Plate® E. coli and Coliform (PPEC or PPECCD) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

k. Charm® Peel Plate® Coliform Count High-Volume (PPCCHV or PPCCCDHV) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

l. Charm® Peel Plate® E. coli and Coliform High-Volume (PPECHV or PPECCDHV) Plate _____

1. Lot #: _____ Exp. Date: _____ _____

m. Brilliant Green Lactose Bile Broth (BGLB): _____ _____

1. Composition:

Peptone or Gelysate	10 g	_____
Lactose	10 g	_____
Oxgall	20 g	_____
Brilliant Green	0.0133 g	_____

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		MS water to make……	1 L	_____
	2. Lot #: _____	Exp. Date: _____		_____
n.	PM Indicator Agar (PMI): _____			_____
	1. Composition:	Beef Extract……	3 g	_____
		Peptone……	5 g	_____
		Tryptone……	1.7 g	_____
		Soytone……	0.3 g	_____
		Dextrose……	5.25 g	_____
		Sodium Chloride……	0.5 g	_____
		Dipotassium Phosphate……	0.25 g	_____
		Polysorbate 80……	1 g	_____
		Bromocresol Purple……	0.06 g	_____
		Agar……	15 g	_____
		MS water to make……	1 L	_____
	2. Lot #: _____	Exp. Date: _____		_____
o.	Buffered Rinse Solution: _____			_____
	1. Composition:	Stock Phosphate Buffer……	1.25 mL	_____
		10% Na Thiosulfate Solution……	5 mL	_____
		Azolectin……	4 g	_____
		Tween 20……	10 g	_____
		MS water to make……	1 L	_____
	2. Weigh hygroscopic Azolectin rapidly and dissolve by heating over boiling water			_____
	3. Date Prepared: _____			_____
p.	Nutrient Broth (NB) (laboratory use only): _____			_____
	1. Composition:	Beef Extract……	3 g	_____
		Peptone……	5 g	_____
		MS water to make……	1 L	_____
	2. Lot #: _____	Exp. Date: _____		_____
q.	Letheen Broth: _____ **(For use with Petrifilm, DO NOT use diluents containing thiosulfate or sodium citrate)**			_____
	1. Composition:	Peptamin……	10 g	_____
		Beef Extract……	5 g	_____
		Lecithin……	0.5 g	_____
		Sorbitan Monooleate……	5 g	_____
		Sodium Chloride……	5 g	_____
		MS water to make……	1 L	_____

2. Lot #: _____ Exp. Date: _____ _____

r. Lauryl Sulfate Tryptose Broth (LST): _____

1. Composition:	Tryptose	20 g	_____
	Lactose	5 g	_____
	Dipotassium Phosphate	2.75 g	_____
	Monopotassium Phosphate	2.75 g	_____
	Sodium Chloride	5 g	_____
	Sodium Lauryl Sulfate	0.1 g	_____
	MS water to make	1 L	_____

2. Lot #: _____ Exp. Date: _____ _____

s. EC-MUG: _____

1. Composition:	Tryptose	20 g	_____
	Lactose	5 g	_____
	Bile Salts Mixture	1.5 g	_____
	Dipotassium Phosphate	4 g	_____
	Monopotassium Phosphate	1.5 g	_____
	Sodium Chloride	5 g	_____
	4-Methylumbelliferyl-β-D-Glucuronide	0.05 g	_____
	MS water to make	1 L	_____

2. Lot #: _____ Exp. Date: _____ _____

t. M-Endo Agar: _____ _____

1. Composition:	Yeast Extract	1.2 g	_____
	Casitone	3.7 g	_____
	Thiopeptone	3.7 g	_____
	Tryptose	7.5 g	_____
	Lactose	9.4 g	_____
	Dipotassium Phosphate	3.3 g	_____
	Monopotassium Phosphate	1 g	_____
	Sodium Chloride	3.7 g	_____
	Sodium Desoxycholate	0.1 g	_____
	Sodium Lauryl Sulfate	0.05 g	_____
	Sodium Sulfite	1.6 g	_____
	Basic Fuchsin	0.8 g	_____
	Agar	15 g	_____
	MS water to make	1 L	_____

1. Lot #: _____ Exp. Date: _____ _____

u. M-Endo Broth: _____

1. Composition:	Yeast Extract	1.5 g	_____
	Casitone	5 g	_____
	Thiopeptone	5 g	_____
	Tryptose	10 g	_____

Lactose	12.5 g	_____
Dipotassium Phosphate	4.375 g	_____
Monopotassium Phosphate	1.375 g	_____
Sodium Chloride	5 g	_____
Sodium Desoxycholate	0.1 g	_____
Sodium Lauryl Sulfate	0.05 g	_____
Sodium Sulfite	2.1 g	_____
Basic Fuchsin	1.05 g	_____
MS water to make	1 L	_____

1. Lot #: _____ Exp. Date: _____ _____

v. Idexx Colilert® _____

1. Lot #: _____ Exp. Date: _____ _____

w. Idexx Colilert®-18 _____

1. Lot #: _____ Exp. Date: _____ _____

x. Idexx Colisure® _____

1. Lot #: _____ Exp. Date: _____ _____

y. Charm® E*Colite _____

1. Lot #: _____ Exp. Date: _____ _____

z. Modified Colitag™ _____

1. Lot #: _____ Exp. Date: _____ _____

29. Medium Preparation _____

a. Media-making utensils of borosilicate glass, stainless steel, or other non-corrosive equipment _____

b. Weigh required amount of dehydrated medium or ingredients _____

c. Combine with required amount MS water, dissolve and mix in a suitable container _____

d. Adjust pH if necessary _____

e. Heat (covered), not under pressure, if necessary, to complete solution (microwave preparation not allowed) _____

f. Restore water as necessary, to compensate for loss due to evaporation _____

g. Distribute into suitable containers so that no part of medium is more than 2.5 cm from any surface _____

1. In general, containers filled no more than half of total volume _____

h. Use suitable container closure and autoclave as necessary _____

30. Prepared Media Storage _____

a. Protect from water loss and light _____

b. Store only screw-capped containers no more than 6 months _____

c. Store prepared Charm PMI plates, no more than 5 days in sealed container at 0.0-4.5°C (tag with date of preparation) _____

d. BGLB broth at room temperature _____

1. Screw capped tubes for 3 months _____

2. Loose (slip) capped tubes for 1 week _____

3. Store in dark _____

e. 3M Petrifilm plate storage _____

1. Store unopened pouches refrigerated or frozen (-30 to 8°C) _____

2. Just prior to use, allow unopened pouches to come to room temperature _____

3. Use before expiration date on package _____

4. After opening, return unused plates to foil pouch, seal pouch by folding and taping/clipping open end shut _____

5. Store opened (re-sealed) pouches at ≤ 25°C _____

6. **Do not refrigerate opened packages.** If laboratory temperature exceeds 25°C, place resealed pouches in a sealable container and store in freezer. Allow plates to acclimate to room temperature before using _____

7. Use Petrifilm plates within one month after opening package (tag with date opened) when storing at lab temperature. If storing in freezer, use within product expiration date _____

f. Pre-dispensed rinse solutions for containers _____

1. Dispense in appropriate volume (20, 50, 100 mL, or other) and sterilize _____

2. Perform quality control checks for volume (100±2 mL) as in item 25.d _____

g. Charm Peel Plate® Storage _____

1. Store unopened packages of Peel Plate® plates at 0-25°C, if refrigerated, allow 30 min to acclimate to room temperature before opening packages _____

2. Use before expiration date on package _____

3. After opening, return unused plates to the foil pouch with desiccant indicator, zip-seal open end shut _____

4. Store opened (re-sealed) packages at 0-25°C _____

5. Check desiccant indicator of Peel Plate® plates before use. Do not use if desiccant has turned white or pink. Do not use if plates are discolored, pink, yellow or brown _____

31. Detergent Suitability Test _____

a. Perform detergent residue test if laboratory uses glass Petri dishes for routine testing _____

b. Detergent is suitable for laboratory use _____

Brand: _____ Brand: _____ _____

c. Test each new brand/lot; maintain records _____

32. Cleaning Pipets (Reusable) _____

a. Discard used pipets in disinfectant _____

b. Rinse in tap water at 15-30°C _____

c. Thoroughly wash with suitable detergent and rinse _____

d. Clean with strong cleaning solution such as acid dairy cleaner as necessary _____

e. Final rinse with MS or DI water _____

f. Test several pieces from each batch (preferably while still wet) for residual acid or alkali with aqueous 0.04% bromothymol blue. If color reaction not dark green to light blue, re-rinse and test again; maintain records _____

33. Cleaning Other Glassware and Apparatus _____

a. Heat to 85°C or disinfect unless pathogens are suspected; then sterilization required prior to washing _____

b. Wash with hot water and suitable detergent and rinse _____

c. Machine washed: (_____) _____

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d. Hand washed: _____ _____

e. Final rinse with MS or DI water _____

f. Test several pieces from each batch (preferably while still wet) for residual acid or alkali with aqueous 0.04% bromothymol blue. If color reaction not dark green to light blue, re-rinse and test again; maintain records _____

SAMPLES

34. Laboratory Requirements _____

a. Section 6 sample requirements _____

1. Record time, date, and temperature of samples when received, and the initial(s), license or permit number or name of the person who received the samples at the laboratory _____

2. Determine sample temperature _____

a. Insert a pre-cooled thermometer into TC (pre-cooling of electronic/ digital thermometer probe is not necessary) _____

b. TC must be at least half the size of the largest test container _____

c. Performed by trained personnel. Maintain records of training _____

3. Finished Product Samples(s) _____

a. Date, time and temperature of collection at the plant or sampling location _____

b. Sample collector's name and license or permit number _____

c. The above information does not need to reside in the laboratory records, but must be available at the same facility _____

4. Producer Universal Sample information required for NCIMS certified laboratories to accept sample to perform regulatory testing as required under the NCIMS program _____

a. Producer identification _____

b. Date of collection at the farm _____

c. Time of collection (Responsibility of the owner of the milk). One of the following options may be used: _____

1. On the sample _____

2. On the records supplied _____

3. Pilot sample (TC) _____

4. In consultation with the state regulatory agency _____

5. Time of collection is not available – use the procedure in current 34.a.7.b _____

d. Non laboratory records - records that are not required to reside in the laboratory: _____

1. Hauler/Sampler name and license/permit number _____

2. Temperature at time of collection at the farm _____

5. Temperature Control (TC) sample is available for each group of sample(s) received at the laboratory. One of the following options may be used: _____

a. Producer Bulk Milk Pick Up Tanker (TC) _____

b. Finished/Packaged Product Sample (TC) _____

c. A single TC per cooler/shipping container shipped from sample depot to the testing lab _____

d. If a TC is not available then any sample in a cooler/shipping container may be used as a TC _____

6. Sample requirements necessary for NCIMS laboratories to accept samples for Section 6 testing _____

a. Producer samples are about ¾ full. Samples too full are not tested _____

b. Samples at the time of receipt by the testing laboratory must be 0.0 to 4.5°C to be accepted for regulatory testing. Liquid samples must not be frozen _____

c. Samples must not be leaking. Do not accept _____

d. Tops of samples must be protected from direct contact with ice _____

e. Unprotected sample(s) must not be submerged in water and/or ice or slush _____

f. If milk sample temperature control exceeds 4.5°C on receipt, do not test microbiologically (samples may be tested if temperature does not exceed 7.0°C and time of receipt is ≤ 3 hours from collection and sample temperature at receipt is no greater than at collection) _____

7. Additional requirements after the samples have been accepted by the testing laboratory _____

   a. Samples stored at 0.0-4.5°C until tested. If samples are frozen, contain ice crystals or exceed 4.5°C, do not test and record as Lab Accident (LA) _____

      1. Samples held at 13°C±1°C for 18±3 hours may be tested for official ESCC _____

   b. Testing of samples to begin no longer than 60 hours from the time the sample was first collected (i.e., producer bulk tank samples or plant finished product samples). If no time of collection is available, use 12:01 AM of the day of collection _____

   c. Remove portions for microbiological analyses first if chemical tests are to be performed, unless superseded by another FDA/NCIMS 2400 form procedure _____

   d. Record date, time and temperature of samples when tested _____

b. Appendix N sample requirements _____

   Refer to App. N GR item 9 _____

35. Sample Bench Sheet Requirements _____

a. Sample collection information: The following information must be readily available for Section 6 producers (item 34.a.4) and finished product samples (item 34.a.3) _____

b. Test information _____

   1. Must show date, time and temperature of samples at the start of analysis and name or initials of the analyst performing the test for each group of samples _____

   2. Test records _____

      a. Bench sheets or records must contain all results (raw and calculated In proper format for tests performed); item 2 _____

      b. Results of all applicable controls for each group of samples must be recorded _____

      c. Plate count procedure controls include: _____

         1. Microbic air density _____

         2. Dilution buffer _____

3. Pipets or pipettor tips _____

4. Agar (when used) _____

5. Temperature of agar (when used) at plating (45±1°C) _____

d. Results of inhibitor tests accompany all plate counts. Inhibitor controls performed and results recorded for each group of samples _____

MISCELLANEOUS

35. Laboratory Practices _____

a. Personnel adequately trained and/or supervised _____

b. Satisfactory participation in annual split samples _____

c. Copies of current, applicable FDA/NCIMS 2400 forms in laboratory _____

d. Copy of written Quality Assurance Plan; required for state central laboratories _____

e. Laboratory management has signed and returned the agreement to abide by the provisions of the NCIMS and the procedures for the Evaluation of Milk Laboratories (EML) _____

f. Laboratory evaluation officer conducted survey unobstructed by laboratory or facility personnel _____

Appendix F: Additional resources for Salmonella, Shiga toxin, and *Salmonella*

Method: FDA Bacteriological Analytical Manual Chapter 5 – *Salmonella*

https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella

Description: culture-based for isolation, various methods for confirmation, Loop-mediated isothermal amplification (LAMP) screening method for various matrices.

Method Type: Can be a standardized method if method attributes (e.g. broth selection and confirmation steps are further defined), needs quality systems to be incorporated; not validated for cannabis

Used in regulatory elsewhere (list): Yes, FDA; many other entities utilize modified BAM protocols

Includes daily/batch QC (list): not included but could be included with ease; process controls should be required to demonstrate acceptable testing parameters/environment (with each batch). Typical *Salmonella* spp. (ATCC) strain(s) would be required to demonstrate acceptable media requirements and satisfactory processing/testing. Negative controls would consist of competitors (i.e. *E. coli, Proteus* spp.) to demonstrate acceptable media quality (follow manufacturer instructions)

Necessitates advanced and/or special training/education: Advanced microbiology experience would be necessary; analyst with previous microbiological experience in food/environmental or clinical testing utilizing culture and molecular techniques would be best suited for this testing

Notes: Culture dependent test; incorporating screening method would be beneficial

Method: NYS DOH Wadsworth LEB-611, Salmonella in Medical Marijuana Products

https://www.wadsworth.org/sites/default/files/WebDoc/NYS%20DOH%20LEB-611-02%2009102018.pdf

Description: culture-based method, isolates were confirmed

Method Type: Standardized method; utilized for the medical marijuana products in New York.

Used in regulatory elsewhere (list): Yes, New York

Includes daily/batch QC (list): Media QC should follow provided parameters and meet vendor requirements; batch QC not listed but should include typical *Salmonella* spp. (ATCC) strain for process control

Necessitates advanced and/or special training/education: Advanced microbiology experience would be necessary; analyst with previous microbiological experience in food/environmental or clinical testing utilizing culture and molecular techniques would be best suited for this testing

Notes: Culture dependent test; does not utilize any molecular testing (see Microbial WG Motion #1). Confirmation of isolates is performed but may not be necessary. The method has been implemented for cannabis matrix; the enrichment process would be useful to explore to combine with molecular testing.

Method: hygiena BAX System Real-time PCR for *Salmonella*

Part KIT2006 (D14306040)

https://www.hygiena.com/wp-content/uploads/2020/09/BAX-Q7-Assay-Kit-Insert-Salmonella-RT-English.pdf

Description: real-time PCR kit

Method Type: Standardized method; does not have incorporated quality systems

Used in regulatory elsewhere (list): Several regulatory bodies have utilized in the past

Includes daily/batch QC (list): not listed but could be easily incorporate into testing infrastructure; matrix controls could be utilized (recommended). *Salmonella* spp. as a PCR positive control and a known negative PCR control (*E. coli*). Enrichment process should include batch controls to maintain media and process efficacy.

Necessitates advanced and/or special training/education: Industry testing friendly; very close to "plug and play" with minimal microbiological training and relatively easy interpretation.

Notes: Vendor specific test that requires testing equipment to perform test.

STEC

Method: hygiena BAX System Real-time PCR STEC Screening Assay

Part KIT2021 (D14642964)

https://www.hygiena.com/wp-content/uploads/2021/04/BAX-Q7-Assay-Kit-Insert-RT-STEC-Suite-EN-rev5.pdf

Description: real-time PCR kit

Method Type: Standardized method; does not have incorporated quality systems

Used in regulatory elsewhere (list): Several regulatory bodies have utilized in the past

Includes daily/batch QC (list): not listed but could be easily incorporate into testing infrastructure; matrix controls could be utilized (recommended). Screening assay would require a Shiga toxin positive *E. coli* strain. The additional panel assays are not necessary to meet the WAC requirement.

Necessitates advanced and/or special training/education: Industry testing friendly; very close to "plug and play" with minimal microbiological training and relatively easy interpretation.

Notes: Vendor specific test that requires testing equipment to perform test.

Method: FDA Bacteriological Analytical Manual Chapter 4a – Diarrheagenic *Escherichia coli*

https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4a-diarrheagenic-escherichia-coli

Description: culture-based for isolation, utilizes molecular methods for screening

Method Type: Can be a standardized method, needs defining for preparation techniques, needs quality systems to be incorporated; not validated for cannabis

Used in regulatory elsewhere (list): not evident based to work group members

Includes daily/batch QC (list): not included but could be included with ease. Shiga toxin positive E. coli strains must be included or molecular screening method. Process/batch controls should be included to demonstrate efficacy. Follow manufacturer instructions for culture media QC

Necessitates advanced and/or special training/education: Advanced microbiology experience would be necessary; analyst with previous microbiological experience in food/environmental or clinical testing utilizing culture and molecular techniques would be best suited for this testing

Notes: Molecular screening test is recommended on both mixed culture and isolated colonies to confirm toxigenic profile/potential

Mycotoxins

Method: Romer Labs ELISA

- **AgraQuant® Total Aflatoxin (CAT No. 10002100/10002101)** https://www.romerlabs.com/shop/agraquant-r-total-aflatoxin-elisa-test/#theme.catalog.product.additional.information.documents.detailed
- **AgraQuant® Ochratoxin (CAT No. 10002102/10002103)** https://www.romerlabs.com/shop/agraquant-r-ochratoxin-elisa-test/#theme.catalog.product.additional.information.documents.detailed

Description: ELISA

Method Type: Standardized method, however, needs quality systems to be incorporated; not validated for cannabis

Used in regulatory elsewhere (list): work group members have utilized kit, also used in other cannabis testing labs in WA

Includes daily/batch QC (list): kit includes controls, frequency not listed

Necessitates advanced and/or special training/education: Good laboratory practices necessary, plate reader assists for interpretation.

Notes: Implementation is relatively easy, very minimal infrastructure necessary. LCMS is alternative method that is used by majority of cannabis labs in WA.