CULTURAL PROCEDURES - GENERAL REQUIREMENTS

[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 16-27C reasonably free from dust and drafts _____

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

d. Microbic density of air ≤ 15 colonies/plate in 15 min exposure, or ≤ 10 colonies/PAC plate in 15 min exposure, if not corrective actions taken _____

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

f. Safe working environment - Refer to OSHA _____

1. Eating and drinking <u>not</u> permitted in laboratory _____

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

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

4. Safety equipment available _____

5. MSDS sheets in laboratory available 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 _____

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 must be clearly documented. 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. Thermometers _____

a. National Institute of Standards and Testing (NIST) traceable thermometer, or equivalent, with certificate. Must be checked 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 0.5C (0-100C) otherwise not more than 1.0C (< 0 or > 100C) _____

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

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

2. Plastic lamination recommended for mercury thermometers _____

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

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 ±1C when checked at temperature(s) of use _____

3. Results recorded/documented and individual devices tagged _____

   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. Records maintained _____

   2. Optionally, a minimum two-day backup power source (battery/electrical) for the temperature monitoring system and/or all required sensors/probes, remote signal device 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 2f above _____

f. Automatic temperature recording instruments, if used, compared weekly against an accurate thermometer, results recorded _____

g. Dial thermometers not used in the laboratory _____

4. Refrigeration (Sample _____) _____

(Reagent _____) _____

a. Size adequate for workload _____

b. Maintains samples at 0-4.4C; 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 -15C 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, calibrated, fixed volume or electronic only _____

1. Pipettors etched with identification (imprinted serial numbers acceptable) and tagged with date accuracy checked _____

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

3. 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 by volume using class A graduated cylinder), records maintained _____

4. 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, records/printouts maintained _____

a. Instrument, printer connected by manufacturer's supplied cable or instrument connected to computer via serial cable _____

b. Instrument and printer (if applicable) connected to 120v/60Hz power _____

c. Reagent kits and Instrument Calibrator kits stored at room temperature _____

1. Lot # _____ Exp. Date _____ _____

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

e. Certificates of Calibration for Reagent Kit and Instrument Calibrator kit maintained in records _____

f. Instrument Validation Guide available _____

g. PCS Pipette Calibration System Procedure, follow manufacturer's Procedure Guide and instrument prompts _____

1. Uncover and insert Blank into the instrument _____

2. Determine which volumes are to be calibrated _____

3. Select the correct Sample Solution and aliquot sufficient amount into working vessel provided _____

4. Using the Pipettor to be verified, aspirate the Sample Solution from the working vessel and deliver it into the Blank seated in the instrument _____

5. When appropriate number of data are collected, press 'End of Run' button _____

6. Record results and file Pipette Calibration Certificate (printout) _____

h. 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) _____

i. PCS Calibration System Validation _____

1. Upon receipt, validate the instrument by following the manufacturer's protocol _____

7. Pipet Containers _____

a. Used 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 G. stearothermophilus (A.K.A. - B. 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 _____

Brand _____ _____

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

Brand _____ _____

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

10. Petri Dish Container _____

a. Used 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 220C) _____

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

d. Records maintained 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 (B. subtilus) strips, include positive control check, results maintained _____

1. Brand: _____ _____

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

12. Sterilization by Dry Heat _____

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

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 and results recorded or downloaded and printed _____

f. Cycle timing checked quarterly and found be accurate, records maintained _____

g. Records maintained 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 maintained.) _____

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, electronic high temperature data loggers can be used 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, temperature readings downloaded and printed _____

h. Temperature indicator used each load _____

i. Performance checked with full load and results recorded weekly using spore (G. stearothermophilus) strips or suspensions, include positive control check, results maintained _____

1. Brand: _____ _____

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

j. Routine maintenance performed and records maintained _____

14. Sterilization by Moist Heat _____

a. Media autoclaved at 120±1C _____

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

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

b. Media autoclaved within 1 hr of preparation _____

c. Dilution buffer autoclaved on same day prepared _____

d. Stoppers or caps slightly loosened to permit passage of steam and air _____

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

f. Autoclave will reach 120±1C 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 in autoclave less than 1 hour _____

15. Incubator and/or Incubator Room _____

(#1 _____) _____

(#2 _____) _____

a. Sufficient size to prevent crowding of interior _____

b. Shelves placed to assure uniform temperature _____

c. 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) _____

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

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

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. All measurements made at room temperature _____

b. Instrument standardized with known buffer solutions _____

1. Three commercially prepared standard solutions used _____

2. Each aliquot used once and discarded _____

3. pH 4, 7 and 10 suggested for linearity and proper function of meter _____

4. Slope determined (95 - 102%) _____ each time meter calibrated, records maintained _____

c. Medium pH recorded each time measured _____

d. Final (after sterilization) pH of each batch of medium determined before use, records maintained _____

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 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 _____

20. Balance _____

a. Electronic only, sensitive to ≥ 0.1g for general laboratory purposes and proper sensitivity for calibrations 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. Checked monthly with weights corresponding to normal use of balance, records maintained _____

d. Checked 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 64C _____

c. Appropriate size for work loads _____

d. Suitable water level maintained _____

22. Mechanical Dilution Bottle Shaker _____

a. Type described in SMEDP, 11th Edition _____

b. Other acceptable _____ _____

23. Microwave Oven for Melting Media _____

a. Analysts instructed to take extreme caution as media expands rapidly at the boiling point _____

24. Microbiologically Suitable (MS) Water _____

a. Type _____ _____

b. System used _____ _____

c. Monthly testing criteria

1. Standard plate count and Petrifilm Aerobic Count films < 1,000 colonies/mL(< 10,000 colonies/mL if stored) _____

2. Total chlorine residual negative, recorded 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 umhos/cm (uSi/cm) at 25C _____

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 0.05 mg/L for each metal and not to exceed 0.1 mg/L total for all metals _____

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

f. Records maintained _____

25. Dilution Buffer and Blanks _____

a. Stock phosphate buffer (Date prepared ___/___/___) _____

1. Prepared in laboratory (34g KH_2PO_4/liter) with MS water _____

2. Purchased commercially prepared (_____) _____

a. Lot No. _____ Exp. Date _____ _____

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

b. Stock $MgCl_2$ Solution, Optional (Date prepared ___/___/___) _____

1. Prepared in laboratory (38g $MgCl_2$/liter or 81.1 g $MgC1_2 \cdot 6H_20$/liter) with MS water _____

2. Purchased commercially prepared (_____) _____

a. Lot No. _____ Exp Date _____ _____

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

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

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

d. Dilution bottles filled 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 graduate 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. Blanks tested 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, records maintained _____

f. Alternatively, commercially prepared dilution buffer blanks used

Brand _____ _____

Lot No. _____ Exp. date _____ _____

1. Volume records maintained as above _____

2. Toxicity checked as above on each new lot received _____

3. Check pH and record _____

g. Records maintained _____

h. Corrective action taken when criteria not met, records maintained _____

26. Reagent Chemicals - of ACS Grade _____

27. Media _____

a. Use dehydrated medium of correct composition _____

1. Stored 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. Discarded if any change is noted in appearance or hydration regardless of manufacturer's expiration date _____

b. Plate Count Agar _____ _____

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 No. _____ Exp. Date _____

c. Petrifilm Aerobic Count (PAC) Plate _____

1. Lot No. _____ Exp. Date _____

d. Violet Red Bile Agar _____ _____

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 No. _____ Exp. Date _____

e. Petrifilm Coliform Count (PCC) Plate _____

1. Lot No. _____ Exp. Date _____

f. Petrifilm High Sensitivity Coliform Count (HSCC) Plate _____

1. Lot No. _____ Exp. Date _____

g. Brilliant Green Lactose Bile Broth _____ _____

1. Composition

Peptone or Gelysate	10 g	_____
Lactose	10 g	
Oxgall	20 g	
Brilliant Green	0.0133 g	
MS water to make	1 L	

2. Lot No. _____ Exp. Date _____

h. PM Indicator Agar _____ _____

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	
Brom Cresol Purple	0.06 g	
Agar	15 g	
MS water to make	1 L	

2. Lot No. _____ Exp. Date _____

i. 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 ___/___/___ _____

j. Nutrient Broth (laboratory use only) _____ _____

1. Composition	Beef Extract	3 g
	Peptone	5 g
	MS water to make	1 L

2. Lot No. _____ Exp. Date _____

k. 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 No. _____ Exp. Date _____

l. Lauryl 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 No. _____ Exp. Date _____

m. 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

_____

2. Lot No. _____ Exp. Date _____

n. 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

_____

2. Lot No. _____ Exp. Date _____

o. Idexx Colilert _____

1. Lot No. _____ Exp. Date _____ _____

p. Idexx Colilert-18 _____

1. Lot No. _____ Exp. Date _____ _____

q. Idexx Colisure _____

1. Lot No. _____ Exp. Date _____ _____

r. Charm E*Colite _____

1. Lot No. _____ Exp. Date _____ _____

28. Medium Preparation _____

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

b. Weigh required amount of dehydrated medium or ingredients _____

c. Combined with required amount MS water, dissolved and mixed in a suitable container _____

d. pH adjusted if necessary _____

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

f. Water restored, as necessary, to compensate for loss due to evaporation _____

g. Distributed 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. Suitable container closures used and autoclaved as necessary _____

29. Prepared Media Storage _____

a. Protected from water loss and light _____

b. Only screw-capped containers kept no more than 6 months _____

c. Prepared Charm PMI plates, kept no more than 5 days in sealed container at 0-4.4C (tag with date of preparation) _____

d. BGB broth at room temperature

1. Screw capped tubes for 3 months _____

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

3. Stored in dark _____

e. Petrifilm plate storage _____

1. Refrigerate unopened packages of Petrifilm plates at or below 8C, if frozen allow 30 min room temperature thaw time before opening packages _____

2. Use before expiration date on package _____

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

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

5. **Do not refrigerate opened packages.** If laboratory temperature exceeds 25C, store resealed pouches of Petrifilm plates in freezer. Allow plates to acclimate to room temperature before using _____

6. 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 described in cultural procedures item 25d _____

30. Detergent Suitability Test _____

a. Detergent residue test performed if laboratory uses glass Petri dishes for routine testing _____

b. Detergent is suitable for laboratory use _____

Brand _____ Brand _____ _____

c. Test each new brand/lot, records maintained _____

31. Cleaning Pipets _____

a. Used pipets discarded in disinfectant _____

b. Rinsed in tap water at 15-30C _____

c. Thoroughly washed with suitable detergent and rinsed _____

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

e. Final rinse with MS water _____

f. Several pieces from each batch tested (preferably while still wet) for residual acid or alkali with aqueous 0.04% bromthymol blue. If color reaction not dark green to light blue, re-rinse and test again. Records maintained _____

32. Cleaning Other Glassware and Apparatus _____

a. Heated to 85C or disinfected unless pathogens suspected; then sterilization required prior to washing _____

b. Washed with hot water and suitable detergent and rinsed _____

c. Machine washed (_____) _____

d. Hand washed _____ _____

e. Final rinse with MS water _____

f. Several pieces from each batch tested (preferably while still wet) for residual acid or alkali with aqueous 0.04% bromthymol blue. If color reaction not dark green to light blue, re-rinse and test again. Records maintained _____

SAMPLES

33. 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 probes is not necessary) _____

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

c. Performed by trained personnel. Records of training maintained. _____

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 33a7b. _____

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 4.4C 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 4.4C on receipt, do not test microbiologically (samples may be tested if temperature does not exceed 7C 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 to 4.4C until tested. If samples are frozen, contain ice crystals or exceed 4.4C, do not test and record as LA. _____

1. Samples held at 13C +/- 1C 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 superceded by another 2400 form procedure _____

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

b. Appendix N sample requirements _____

Refer to Appendix N General Requirements Item 9 _____

34. Sample Bench Sheet Requirements _____

a. Sample collection information: The following information must be readily available for Section 6 producer (see item 33a4) and finished product samples (see item 33a3) _____

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), see 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±1C) _____

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 2400 series survey 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 _____

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