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 <u>not</u> 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 _____

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 _____

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	_____

		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: (_____) _____

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 _____