

# CULTURAL PROCEDURES-GENERAL REQUIREMENTS

[Unless otherwise stated all tolerances are  $\pm 5\%$ ]

## 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  $\leq 15$  colonies/SPC or RAC plate,  $\leq 10$  colonies/PAC plate or  $\leq 5$  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 \_\_\_\_\_

- 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, 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  $\pm 5\%$  of specified delivery volume (by weight, or if  $\geq 1.0$  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  $\pm 5\%$  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  $\geq 170^{\circ}\text{C}$  for  $\geq 2$  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  $120\pm 1^\circ\text{C}$  \_\_\_\_\_

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  $120\pm 1^\circ\text{C}$  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 ( $\geq 15$  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 in rehydrated PAC plates or PPAC plates must not lose more than 15% weight after 48 hours of incubation. Agar in rehydrated RAC plates must not lose more than 15% weight after 24 hours of 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. Lethen 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 [Not 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, 3M™ Petrifilm™ Aerobic Count, 3M™ Petrifilm™ Rapid Aerobic Count, or Charm 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 0.05 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. Dilution Buffer and Blanks**

- a. Stock phosphate buffer (Prep. Date: \_\_\_\_\_) \_\_\_\_\_
  - 1. Prepare in laboratory (34 g  $\text{KH}_2\text{PO}_4/\text{L}$ ) with MS water; OR \_\_\_\_\_
  - 2. Purchase commercially prepared (\_\_\_\_\_ ) \_\_\_\_\_
    - a. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_ \_\_\_\_\_
  - 3. Place in small containers ( $\leq 100$  mL), autoclave and store in refrigerator \_\_\_\_\_
- b. Stock  $\text{MgCl}_2$  Solution, Optional (Prep. Date: \_\_\_\_\_) \_\_\_\_\_
  - 1. Prepare in laboratory (38 g  $\text{MgCl}_2/\text{L}$  or 81.1 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}/\text{L}$ ) with MS water; OR \_\_\_\_\_
  - 2. Purchase commercially prepared (\_\_\_\_\_ ) \_\_\_\_\_
    - a. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_ \_\_\_\_\_
  - 3. Place in small containers ( $\leq 100$  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  $\text{MgCl}_2/\text{L}$  of MS water \_\_\_\_\_
- d. Fill dilution bottles to contain  $99 \pm 2$  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 \_\_\_\_\_

**26. Reagent Chemicals – of ACS Grade** \_\_\_\_\_

**27. 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 (PPEC) Plate \_\_\_\_\_
1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_ \_\_\_\_\_
- j. Charm® Peel Plate® Coliform Count High Volume Sensitivity (PPECHVS) Plate \_\_\_\_\_
1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_ \_\_\_\_\_
- k. 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: \_\_\_\_\_ \_\_\_\_\_

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

2. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

m. 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: \_\_\_\_\_

n. 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: \_\_\_\_\_

o. Lethen 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: \_\_\_\_\_

p. 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: \_\_\_\_\_

q. 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- $\beta$ -D-Glucuronide	0.05 g	_____
	MS water to make	1 L	_____

2. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

r. 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: \_\_\_\_\_

s. 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: \_\_\_\_\_

t. Idexx Colilert® \_\_\_\_\_

1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

u. Idexx Colilert®-18 \_\_\_\_\_

1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

v. Idexx Colisure® \_\_\_\_\_

1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

w. Charm® E\*Colite \_\_\_\_\_

1. Lot #: \_\_\_\_\_ Exp. Date: \_\_\_\_\_

## 28. 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 \_\_\_\_\_

**29. 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  $\leq 25^{\circ}\text{C}$  \_\_\_\_\_
  - 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 3M 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. Refrigerate unopened packages of Charm Peel Plate® plates at or below 8°C; if frozen, 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 or below 8°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 \_\_\_\_\_

**30. 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 \_\_\_\_\_

**31. 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 water \_\_\_\_\_
- f. Test several pieces from each batch (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; maintain records \_\_\_\_\_

**32. 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 water \_\_\_\_\_
- f. Test several pieces from each batch (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; maintain records \_\_\_\_\_

**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. 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 33.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 \_\_\_\_\_

### 34. Sample Bench Sheet Requirements \_\_\_\_\_

a. Sample collection information: The following information must be readily available for Section 6 producers (item 33.a.4) and finished product samples (item 33.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 \_\_\_\_\_