DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

MILK LABORATORY EVALUATION FORM

LABORATORY		
LOCATION		LAB #
DATE	X = DEVIATION O = NOT USED	U = UNDETERMINED NA = NOT APPLICABLE

DAIRY WATERS
[Unless otherwise stated all tolerances are ±5%]

1. Laboratory Requirements ..... _____
- a. CP, items 33 & 34 ..... _____
- b. Sample volume sufficient to assure 100 mL for testing sufficient air space for mixing (about ¾ full), if completely filled do not accept ..... _____
- c. Transported and maintained at 0-4.4C (temperature control [TC] required) ..... _____
- d. If samples are not refrigerated, transit not to exceed 6 hours (TC not required) ..... _____
- e. Transit time does not exceed 30 hours ..... _____
- f. Samples examined within 30 hours of collection or within 2 hours of receipt (item 1d) ..... _____

APPARATUS

2. CP (see items 1 - 32, as necessary) ..... _____

3. Sample Containers ..... _____
- a. Borosilicate glass, plastic bottles or bags ..... _____
- b. Sterile, containing 0.1 mL of 10% Sodium Thiosulfate ..... _____
- c. Holds sufficient sample with air space for all necessary bacterial tests ..... _____
- d. Maintains sample uncontaminated ..... _____

4. Incubator 35±0.5C (Make/Model _____) ..... _____
- a. See CP item 15 for incubator requirements ..... _____

5. Fermentation Tubes/Bottles ..... _____
- a. Sufficient size to conform with requirements for media, durham tube and sample ..... _____

6. Inoculation Equipment ..... _____
- a. Sterilized loops of at least 3 mm diameter, 22-24 gauge nichrome, chromel or platinum-iridium wire ..... _____
- b. Disposable dry heat-sterilized hardwood applicator sticks, 0.2 to 0.3 cm in diameter and a minimum of 2.5 cm longer than the fermentation tubes ..... _____
- c. Inoculating needle ..... _____

7. Vacuum source with trap ..... _____

8. Membrane filter funnel Brand _____ ..... _____
- a. Free from defects that may interfere with function ..... _____
- b. Sterilizable ..... _____
- c. Marked at 100 mL, or pre-marked checked and adjusted, using a 100 mL Class A graduate cylinder ..... _____

9. Membrane cellulose filters, 47 mm, 0.45 µM (±0.02 µM), sterilized ..... _____
Brand _____ Lot # _____ ..... _____

10. Absorbent pads, sterilized Brand _____ ..... _____

11. Forceps ..... _____
- a. Round tipped, with smooth surface ..... _____

12. Culture (Petri) dishes (for MF)
Brand _____ Size _____ ..... _____
- a. Sterile with plastic, tight fitting covers ..... _____

13. Microscope and Lamp
Brand _____ Model _____ ..... _____
- a. Binocular, wide field, 10x oculars ..... _____
- b. Fluorescent light, adjacent, above, perpendicular to filter plane ..... _____
- c. Other optical device giving equivalent results ..... _____

CULTURE MEDIA

14. Storage of media ..... _____
- a. See CP item 27 for media and storage requirements ..... _____
- b. MF Media
  1. Store in dark at 0-4.4C ..... _____
  2. Broth medium used within 96 hr Date prep. _____ ..... _____
  3. Plates kept no more than 1 week in a sealed container at 0-4.4C Date prep. _____ ..... _____

TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP
BY MULTIPLE-TUBE FERMENTATION TECHNIQUE

15. Presumptive Test ..... _____
- a. Lauryl Tryptose Broth ..... _____
  1. Before inoculating arrange tubes in order and label, or otherwise identify ..... _____
  2. Shake samples vigorously 25 times in a 30 cm arc in 7 sec before removing test portion ..... _____
  3. Remove test portions (100 mL total) within 3 min ..... _____
  4. Inoculate ten (10) fermentation tubes with 10 mL of sample or five (5) tubes with 20 mL with double strength LST or one bottle with 100 mL double strength LST ..... _____
  5. Incubate tubes at 35±0.5C for 24±2 hours ..... _____
  6. Examine tubes for gas - any gas is considered presumptive positive ..... _____
  7. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) ..... _____
  8. Re-examine tubes for gas production after 48±3 hours ..... _____
  9. Record presence or absence of gas at each examination ..... _____
  10. Any gas produced by 24 or 48 hr is considered positive for the Presumptive Test ..... _____
  11. No gas after 48 hr is Not Found (NF) for the Test ..... _____
  12. Do not report gas production after 51 hr of incubation ..... _____
  13. Promptly submit all presumptive positive tubes showing gas production at 24 or 48 hr to the Confirmed Test ..... _____

16. Confirmed Test ..... _____
- a. Brilliant Green Lactose Bile Broth
  1. Gently shake presumptive positive tube ..... _____
  2. Transfer (loop or stick) portion of positive broth to BGLB broth ..... _____
  3. Incubate tubes at 35±0.5C for 24±2 hr ..... _____
  4. Examine tubes for gas — any gas is considered positive ..... _____

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5. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) .....
6. Re-examine tubes for gas production after 48 hours .....
7. Record presence or absence of gas at each examination .....
8. Any gas produced by 24 or 48 hr is considered positive for the Confirmed Test .....
9. No gas after 48 hr is Not Found (NF) for the Test .....
10. Do not report gas production after 51 hr of incubation .....

17. Reporting .....

a. Report results of fermentation tubes that confirm as positive, reported as MPN/100 mL (≥1.1/100 mL if 10 tubes used or ≥2.2/100 mL if 5 tubes used), or as ≥1.1/100 mL if 100 mL presence/absence test used .....

b. If one or more tubes turbid with no gas production, invalidate the sample and request a re-sample from the same point source for heterotrophic plate count .....

c. Interpretation for multiple tubes: Not Found (NF) is < 1.1 (or < 2.2)/100 mL and Positive is ≥1.1 (or ≥2.2)/100 mL .....

TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP

BY MEMBRANE FILTRATION TECHNIQUE

18. Filtration .....

a. Place (with alcohol flamed forceps, item 11) sterile membrane filter (item 9) on porous plate, secure funnel .....

b. Pour 100 mL test sample into funnel (item 8)and apply vacuum .....

c. After test volume has been filtered, rinse funnel by filtering 3 volumes of 20-30 mL of sterile buffered water .....

d. Turn off vacuum and remove filter with sterile (alcohol flamed) forceps .....

e. M-endo Broth .....

1. Sterile pad (item 10) placed in culture dish .....
2. Saturate pad with 2.0 mL of M-endo Medium, CP item 27n .....
3. Allow to stand a few minutes before pouring off excess .....
4. Prepared filter rolled (grid side up) onto pad slowly to avoid trapping air bubbles, do not drag across side of plate .....

f. M-endo Agar .....

1. Use culture dish previously prepared (CP item 27m) .....
2. Prepared filter placed on agar with rolling motion to avoid trapping air bubbles .....

19. Incubation .....

a. In saturated humidity, with dish inverted .....

b. At 35±0.5C for 21±1 hr .....

20. Counting .....

a. Count all sheen colonies as typical coliforms and dark suspect colonies as atypical coliforms, keep separate counts of each morphological type until confirmed .....

b. Confirm 10% up to a maximum of 10 isolated colonies, with representative proportions of each colony type .....

21. Confirmation Test .....

a. Make serial transfers of colonies to individual LST and then to BGLB tubes using the same transfer needle/stick .....

b. Incubate tubes at 35±0.5C for 24±2 hr .....

c. Examine tubes for gas .....

1. LST tubes with gas must be transferred to fresh BGLB tubes if the original BGLB tubes show no gas .....

d. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) .....

e. Re-examine tubes for gas production after 48 hours .....

f. Record presence or absence of gas at each examination .....

g. Any gas produced in BGLB tubes by 24 or 48 hrs is considered positive for the Confirmation Test .....

h. No gas after 48 hr is Not Found (NF) for the Test .....

i. Do not report gas production after 51 hr of incubation .....

22. Reporting .....

a. Report confirmed colony count/100 mL .....

b. Invalidate all samples with confluent growth or TNTC, and request a re-sample from the same point source for heterotrophic plate count .....

c. Interpretation: Not Found (NF) is <1/100 mL and Positive is ≥1/100 mL .....

HETEROTROPHIC BACTERIA

STANDARD PLATE COUNT METHOD

23. Heterotrophic Plate Count Method .....

a. Plate samples as in SPC, items 2-10, 13 and 14 .....

b. Incubate at 35±0.5C for 48±3 hours .....

c. Count as in SPC item 16-17 .....

d. Report counts as in SPC item 20 .....

e. Record as "Heterotrophic Plate Count/mL at 35C" .....

f. Interpretation: Negative if <500 CFU/mL and Positive if ≥500 CFU/mL .....

CHROMOGENIC SUBSTRATE (MMO-MUG)

PRESENCE - ABSENCE SCREENING TEST FOR DAIRY WATERS

*(SOURCE WATER SUPPLIES **ONLY**)*

24. Materials .....

a. Color comparator .....

b. Sterile borosilicate glass or clear plastic bottles to contain 100 mL sample with sufficient air space for mixing (about ¾ full) .....

c. MMO-MUG substrate, see CP item 27o .....

d. Quality control procedures conducted on each lot of substrate received, as recommended by manufacturer, test by spiking with known coliform, records maintained .....

25. Procedure .....

a. Aseptically add pre-weighed MMO-MUG substrate to 100 mL of water sample .....

b. Optionally, add 100 mL sample to the MMO-MUG substrate in a sterile container provided by the manufacturer .....

c. Aseptically cap and mix thoroughly by inverting 25 times to dissolve reagent (does not completely dissolve) .....

d. Incubate at 35±0.5C for a **minimum** of 24 hours, not to exceed 28 hours .....

e. Examine containers for the production of yellow color .....

26. Interpretation .....

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DAIRY WATERS

[Unless otherwise stated all tolerances are ±5%]

a. If no yellow color is observed .....
1. Read sample as Not Found (NF) for total coliforms .....
2. Report as total coliform Not Found (NF) in 100 mL sample: <1/100 mL .....

b. If yellow color present .....
1. Gently invert container several times until color is uniformly dispersed through the sample .....
2. Compare yellow color to color comparator dispersed into the **SAME** type of sample container .....
3. If color is equal to or greater than that of the color comparator, sample reported as Positive for total coliforms .....
4. If color is obvious but less than the comparator, sample reported as Not Found (NF) .....
5. Report as total coliforms present in 100 mL sample: ≥1/100 mL .....

CHROMOGENIC SUBSTRATE (MMO-MUG)

MULTIPLE TUBE PROCEDURE FOR THE PRESENCE OF TOTAL COLIFORMS

*(SOURCE WATER SUPPLIES **ONLY**)*

27. Materials (see items 24 a-d) .....

28. Procedure .....

a. Before transferring sample portions arrange tubes in order and identify .....
b. Shake samples vigorously 25 times in a 30 cm arc in 7 sec .....
c. Aseptically add pre-weighed MMO-MUG substrate to 100 mL sample .....
d. Optionally, add 100 mL of sample to container with MMO-MUG substrate provided by manufacturer .....
e. Aseptically cap and mix thoroughly by inverting 25 times to dissolve reagent (does not completely dissolve) .....
f. Remove test portions (100 mL total) within 3 minutes .....
g. Transfer 20 mL of sample/reagent mixture to five tubes, or 10 mL to ten tubes .....
h. Optionally, transfer 100 mL of mixed (see item 28b) sample to 10 tubes containing pre-dispensed MMO-MUG reagent provided by manufacturer .....
i. Incubate tubes at 35±0.5C for a **minimum** of 24 hours, do not to exceed 28 hours .....
j. Examine tubes for the development of yellow color .....
1. Mix tubes to uniformly distribute yellow color .....
2. Compare tubes to color comparator tube (**SAME** size and type as MPN tubes) .....
3. Tubes with color equal to or greater than color comparator tube recorded as Positive .....
4. Tubes with obvious color but less than comparator, sample reported as Not Found (NF) .....

29. Reporting .....

a. If all tubes show no color, report as Not Found (NF): <1.1/100 mL .....
b. If one or more tubes show yellow color (see 28j) report as Positive: MPN/100 mL .....

CHROMOGENIC SUBSTRATE PRESENCE (XGAL-MUG)

ABSENCE SCREENING TEST FOR DAIRY WATERS

*(SOURCE WATER SUPPLIES **ONLY**)*

30. Materials .....

a. E*Colite substrate, see CP item 27p .....
b. Quality control procedures conducted on each lot of substrate received, as recommended by manufacturer, test by spiking with known coliform, records maintained. .....

31. Procedure .....

a. Add water sample to the E*Colited substrate .....
1. Tear perforated strip .....
2. Open bag by pulling white tabs .....
3. Aseptically pour 100 mL of water sample into bag (do not touch inside of bag) .....
4. Flatten bag to remove air .....
5. Twirl bag 2 - 3 times around twister wires to form a leak proof seel .....
6. Fold twisters around back of bag .....
7. Shake bag 25 times in 7 seconds to dissolve sodium thiosulfate tablet, if present .....
8. Continue rolling to build pressure in water compartment .....
9. Maintain pressure on rolled area and push water through first seal into powder section of bag **ONLY** .....
10. Shake bag 25 times in 7 seconds to completely dissolve powder in water (push mixture against bag sides to pull apart any remaining seal) .....

b. Place sealed bag in 35C water bath for 10 minutes .....
c. Transfer to 35±0.5C incubator for 28 hours .....
d. Examine bags for the production of blue or blue/green color, or blue color in corners of bag .....

32. Interpretation .....

a. If yellow color is observed: .....
1. Record sample as Not Found (NF) for total coliforms .....
2. Report as total coliform Not Found (NF) in 100 mL sample: < 1/100 mL .....

b. If blue or blue/green (or blue in corners) color observed: .....
1. The sample is Positive for total coliforms .....
2. Report as total coliforms present in 100 mL sample: ≥1/100 mL .....

MISCELLANEOUS

33. Copy of current in-use edition of *Standard Methods for the Examination of Water and Wastewater* in laboratory .....