

**DAIRY WATERS
(Coliform Group)
IMS #24**

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

1. Laboratory Requirements

- a. CP, items 33 & 34
- b. Sample volume sufficient to assure 100 mL for testing sufficient air space for mixing (about $\frac{3}{4}$ full), if completely filled do not accept
- c. Transported and maintained at 0.0-4.5°C (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 1.d)

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 $35 \pm 0.5^\circ\text{C}$ (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 graduated 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 0.0-4.5°C
 - 2. Broth medium used within 96 hours Date prep. _____
 - 3. Plates kept no more than 1 week in a sealed container at 0.0-4.5°C
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 1 ft 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±0.5°C 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 hours)
 - 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 hours is considered positive for the Presumptive Test
 - 11. No gas after 48 hours is Not Found (NF) for the Test

- 12. Do not report gas production after 51 hours of incubation _____
- 13. Promptly submit all presumptive positive tubes showing gas production at 24 or 48 hours 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±0.5°C for 24±2 hours _____
 - 4. Examine tubes for gas - any gas is considered positive _____
 - 5. Return negative tubes (no gas) to incubator and incubate an additional 24 hours (total of 48±3 hours) _____
 - 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 hours is considered positive for the Confirmed Test _____
 - 9. No gas after 48 hours is Not Found (NF) for the Test _____
 - 10. Do not report gas production after 51 hours 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 mL in 10 tubes or 20 mL in 5 tubes are used) or ≥ 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 < 1.1/100 mL and Positive is ≥ 1.1/100 mL; for presence/absence, NF is < 1/100 mL and Positive is ≥ 1/100 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 27.n

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 27.m)

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 $35 \pm 0.5^\circ\text{C}$ for 21 ± 1 hours

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 $35\pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours
- 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 hours (total of 48 ± 3 hours)
- 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 hours is considered positive for the Confirmation Test
- h. No gas after 48 hours is Not Found (NF) for the Test
- i. Do not report gas production after 51 hours 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 $\geq 1/100$ 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 $35\pm 0.5^{\circ}\text{C}$ for 48 ± 3 hours
- c. Count as in SPC items 16 and 17
- d. Report counts as in SPC item 19

- e. Record as "Heterotrophic Plate Count/mL at 35°C" _____
- f. Interpretation: Negative if < 500 CFU/mL and Positive if ≥ 500 CFU/mL _____

**CHROMOGENIC SUBSTRATE (MMO-MUG) PRESENCE - ABSENCE TEST
FOR DAIRY WATERS (SOURCE WATER SUPPLIES ONLY)**

24. Materials _____

- a. Sterile non-fluorescent borosilicate glass or clear plastic bottles to contain 100 mL sample with sufficient air space for mixing (about ¾ full) _____
- b. Color comparator (required for Colilert and Colilert-18) _____
- c. Commercially prepared substrate used _____
 - 1. Colilert (see Cultural Procedures, item 27.o) _____
 - 2. Colilert-18 (see Cultural Procedures, item 27.p) _____
 - 3. Colisure (see Cultural Procedures, item 27.q) _____
- d. Suitability test conducted on each lot of substrate received, by spiking with known coliform; records maintained _____
- e. Water Bath, circulating, maintains 35±0.5°C; records maintained during periods of use (required for Colilert-18) _____

25. Procedure _____

- a. Aseptically add pre-weighed substrate to 100 mL of the water sample _____
- b. Optionally, add 100 mL of sample to the 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. For Colilert-18, thermally equilibrate test solution for 20 min in a 35±0.5°C circulating water bath and then continue incubation in water bath or dry incubator for a total of 18 hours (minimum), not to exceed 22 hours _____
- e. For Colilert and Colisure, incubate at 35±0.5°C in water bath or dry incubator for a **minimum** of 24 hours, not to exceed 28 hours for Colilert, 48 hours for Colisure _____
- f. Examine containers for the production of color change _____

26. Interpretation and Reporting

a. Colilert and Colilert-18

1. If no yellow color is observed

- a. Record test result as Not Found (NF) for total coliform
- b. Report as total coliform Not Found (NF) in 100 mL sample:
< 1/100 mL

2. If yellow color present

- a. Gently invert container several times until color is uniformly dispersed through the sample
- b. Compare yellow color to color comparator dispersed into the **SAME** type of sample container
- c. If color is equal to or greater than that of the color comparator, record test result as Positive (POS) for total coliform
- d. Report as total coliform Present in 100 mL sample: $\geq 1/100$ mL
- e. If yellow color is obvious but less than the comparator, record test result as Not Found (NF) for total coliform; report as for no yellow color above (26.a.1.b)

b. Colisure

1. If no red or magenta color is observed

- a. Record test result as Not Found (NF) for total coliform
- b. Report as total coliform Not Found (NF) in 100 mL sample:
< 1/100 mL

2. If red or magenta color present

- a. Gently invert container several times until color is uniformly dispersed through the sample
- b. If red or magenta color is present, record test result as Positive for total coliform
- d. Report as total coliform Present in 100 mL sample: $\geq 1/100$ mL

**CHROMOGENIC SUBSTRATE (MMO-MUG) MULTIPLE TUBE PROCEDURE
FOR THE PRESENCE OF TOTAL COLIFORMS (SOURCE WATER SUPPLIES ONLY)**

27. Materials

- a. Sterile non-fluorescent borosilicate glass or clear plastic tubes 10 mL or 20 mL capacity
- b. See items 24.b-e

28. Procedure

- a. Before transferring sample portions arrange tubes in order and identify
- b. Shake sample vigorously 25 times in 7 sec with a 1 ft movement prior to adjusting to test volume
- c. Aseptically add pre-weighed substrate to 100 mL sample
- d. Optionally, add 100 mL of sample to container with 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 min
- g. Transfer 20 mL of sample/reagent mixture to five (5) tubes, or 10 mL to ten (10) tubes
- h. Optionally, transfer 100 mL of mixed (see item 28b) sample to 10 tubes containing pre-dispensed substrate provided by manufacturer
- i. For Colilert-18, thermally equilibrate test solution for 20 min in a 35±0.5°C circulating water bath and then continue incubation in water bath or dry incubator for a total of 18 hours (minimum), not to exceed 22 hours
- j. For Colilert and Colisure, incubate at 35±0.5°C in water bath or dry incubator for a **minimum** of 24 hours, not to exceed 28 hours for Colilert, 48 hours for Colisure
- k. Examine tubes for the development of color change

29. Interpretation

- a. Colilert and Colilert-18
 - 1. Mix tubes to uniformly distribute yellow color

- 2. Compare tubes to color comparator tube (**SAME** size and type) _____
- 3. Record test result of tubes without color or obvious yellow color but less than comparator as Not Found (NF) _____
- 4. Record test result of tubes with yellow color equal to or greater than color comparator tube as Positive (POS) _____
- b. Colisure _____
 - 1. Mix tubes to uniformly distribute red or magenta color _____
 - 2. Record test result of tubes without red or magenta color as Not Found (NF) _____
 - 3. Record test result of tubes with red or magenta color as Positive (POS) _____

30. Reporting _____

- a. If all tubes test Not Found (see 29.a.3 or 29.b.2), report as Not Found (NF): < 1.1/100 mL _____
- b. If one or more tubes test Positive (see 29.a.4 or 29.b.3), report as Positive: ≥ 1.1/100 mL _____

CHROMOGENIC SUBSTRATE PRESENCE (XGAL - MUG) PRESENCE - ABSENCE TEST FOR DAIRY WATERS (SOURCE WATER SUPPLIES ONLY)

31. Materials _____

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

32. Procedure _____

- a. Add water sample to the E*Colite 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 seal _____
 6. Fold twisters around back of bag _____
 7. Shake bag 25 times in 7 sec 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 sec to completely dissolve powder in water (push mixture against bag sides to pull apart any remaining seal) _____
- b. Place sealed bag in 35°C water bath for 10 minutes _____
 - c. Transfer to 35±0.5°C incubator for 28 hours _____
 - d. Examine bags for the production of blue or blue/green color or blue color in corners of bag _____

33. Interpretation

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

MISCELLANEOUS

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