DAIRY WATERS

(Coliform Group and *Escherichia coli*)
[*E. coli* verification required only on source water]
IMS #24

[Unless otherwise stated all tolerances are ±5%]

1. Laboratory Requirements _____

a. Cultural Procedures (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 0.0-4.5°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±0.5°C (Make/Model: _____) _____

a. See CP item 15 for incubator requirements _____

5. Water Bath, 35±0.5°C (Make/Model: _____) _____

a. Circulating and thermostatically controlled _____

b. Maintain sufficient water depth _____

6. Water Bath, 44.5±0.2°C (Make/Model: _____)
[Required for EC-MUG] _____

a. Circulating and thermostatically controlled _____

b. Maintain sufficient water depth _____

7. Water Bath, 44.5±0.5°C (Make/Model: _____) _____
[Only for use with item 31f]

a. Circulating and thermostatically controlled _____

b. Maintain sufficient water depth _____

8. Fermentation Tubes/Bottles _____

a. Sufficient size to conform with requirements for media, Durham tube and sample _____

b. Tubes and bottles used for EC-MUG broth and chromogenic substrate methods do not autofluoresce _____

9. 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 _____

d. Sterile disposable plastic loops _____

e. Commercial pre-sterilized cotton swabs on wooden sticks, 0.2 to 0.3 cm in diameter and a minimum of 2.5 cm longer than the fermentation tubes _____

10. Long Wavelength UV Light (365 – 366 nm) _____

a. 6 watts _____

b. Keep clean _____

11. Vacuum Source with Trap _____

12. Membrane Filter (MF) 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 _____

13. Membrane Cellulose Filters, 47 mm, 0.45 µM (±0.02 µM), Sterilized _____

Brand: _____ Lot #: _____ _____

14. Absorbent Pads, Sterilized, Brand: _____ _____

15. Forceps _____

a. Round tipped, with smooth surface _____

16. Culture (Petri) Dishes (for MF), Brand: _____ **Size:** _____ _____

a. Sterile with plastic, tight fitting covers _____

17. 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

18. Storage of Media _____

a. 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.: _____ _____

19. Media Quality Control _____

a. See CP item 27 for media composition _____

b. Suitability test conducted on each new lot of commercially prepared and/or each new batch of laboratory prepared media by spiking with known coliform or *E. coli*, as applicable; records maintained _____

TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP AND *E. coli* BY MULTIPLE-TUBE FERMENTATION TECHNIQUE

20. Presumptive Test _____

a. Double Strength Lauryl Sulfate Tryptose Broth (DS-LST) _____

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 containing 10 mL DS-LST or five (5) tubes containing 20 mL DS-LST or one bottle containing 100 mL DS-LST with equal volume of sample _____

5. Incubate tubes at 35±0.5°C for 24±2 hours _____

6. Examine tubes for gas - any gas is considered Presumptive Positive and must be transferred to BGLB (all tests) and EC-MUG broth (if performing *E. coli* testing) _____

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 _____

    a. Tubes showing no gas, but showing evidence of growth (turbid) can be promptly submitted to the Confirmation Test (item 21). Do not report until after completion of confirmation _____

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 Confirmation Test _____

21. Total Coliform Confirmation Test _____

a. Brilliant Green Lactose Bile Broth (BGLB) _____

1. Gently shake presumptive positive tube (item 20.a.13) _____

2. Transfer (item 9) 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 Total Coliform _____

9. No gas after 48 hours is Not Found (NF) for Total Coliform _____

10. Do not report gas production after 51 hours of incubation _____

22. *E. coli* Verification Test _____

a. EC-MUG Broth _____

1. Gently shake presumptive positive tube(s) (item 20.a.13) _____

2. Transfer (item 9) portion of positive broth to EC-MUG broth _____

a. If using the same apparatus to transfer to both BGLB and EC-MUG, transfer to EC-MUG first then to BGLB _____

3. Incubate tubes at 44.5±0.2°C for 24±2 hours (item 6 only) _____

a. Place tubes in water bath within 30 min of inoculation _____

4. Examine tubes exhibiting growth for fluorescence using Long-Wavelength UV light (item 10) _____

5. Record presence or absence of fluorescence _____

6. Bright blue fluorescence after incubation is considered positive for *E. coli* _____

7. No fluorescence after incubation is Not Found (NF) for *E. coli* _____

23. Recording and Reporting _____

a. If one or more DS-LST tubes are turbid with no gas production and confirmation in BGLB yields no gas production, invalidate the sample and request a re-sample from the same point source for heterotrophic plate count. [If history has shown a sample source to repeatedly yield growth/turbidity in LST that does not confirm, the lab may test for Heterotrophic Plate Count at the same time as testing the same sample by the Multiple Tube Fermentation technique as a usual practice.] _____

b. Record results of fermentation tubes that confirm positive in BGLB as MPN Total Coliform/100 mL (≥ 1.1/100 mL if 10 tubes of 10 mL or 5 tubes of 20 mL are used) or ≥ 1 Total Coliform/100 mL if 100 mL presence/absence test used _____

c. Record results of fermentation tubes that confirm positive in EC-MUG as MPN *E. coli*/100 mL (≥ 1.1/100 mL if 10 tubes of 10 mL or 5 tubes of 20 mL are used) or ≥ 1 *E. coli*/100 mL if 100 mL presence/absence test used _____

d. 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

24. Filtration _____

a. Place (with alcohol flamed forceps, item 15) sterile membrane filter (item 13) on porous plate, secure funnel _____

b. Pour 100 mL test sample into funnel (item 12) 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 14) placed in culture dish _____

2. Saturate pad with 2.0 mL of M-Endo Broth, CP item 27.r _____

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.q) _____

2. Prepared filter placed on agar with rolling motion to avoid trapping air bubbles _____

25. Incubation _____

a. In saturated humidity, with dish inverted _____

b. At 35±0.5°C for 21±1 hour _____

26. 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 _____

27. Total Coliform Confirmation Test _____

a. Make serial transfers of colonies to individual LST and then to BGLB tubes using the same transfer apparatus (item 9) _____

b. Incubate tubes at 35±0.5°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 _____

28. *E. coli* Verification Test

a. EC-MUG Broth _____

1. Transfer (item 9) portion of each target colony to EC-MUG broth _____

   a. If using the same apparatus to transfer to LTB, BGLB and EC-MUG, Transfer to LTB first, then EC-MUG then to BGLB _____

2. Incubate tubes at 44.5±0.2°C for 24±2 hours (item 6 only) _____

   a. Place tube in water bath within 30 min of inoculation _____

3. Examine tubes exhibiting growth for fluorescence using Long-Wavelength UV light (item 10) _____

4. Record presence or absence of fluorescence _____

5. Bright blue fluorescence after incubation is considered positive for *E. coli* _____

6. No fluorescence after incubation is Not Found (NF) for *E. coli* _____

29. 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

30. Heterotrophic Plate Count Method _____

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

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

c. Count as in SPC item 16 _____

d. Report counts as in SPC item 19.a _____

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

f. Interpretation: Not Found (NF) if < 500 CFU/mL and Positive if ≥ 500 CFU/mL _____

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

31. 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® (CP item 27.s) _____

2. Colilert®-18 (CP item 27.t) (see 32.d) _____

3. Colisure® (CP item 27.u) _____

d. Suitability test conducted on each lot of substrate received, by spiking with known coliform; maintain records _____

e. Water Bath, circulating, maintains 35±0.5°C or; maintain records during periods of use (required for Colilert-18) _____

f. Water Bath, circulating, maintains 44.5±0.5°C (item 6 or 7); maintain records during periods of use (optional for Colilert-18; see item 32.d) _____

32. 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 shaking 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 or alternatively 7-10 min (not to exceed 10 min) in a 44.5±0.5°C circulating water bath (item 6 or 7), and then continue incubation in water bath or dry incubator (35±0.5°C) 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 _____

g. Examine containers that exhibit color change for fluorescence _____

33. 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: ≥ 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 (33.a.1.b) _____

3. Place yellow containers under Long-Wavelength UV light (item 10) _____

a. If the container fluoresces, record test result as Positive (POS) for *E. coli* _____

b. Report as *E. coli* Present in 100 mL sample: ≥ 1/100 mL _____

c. If container does not fluoresce, record test result as Not Found (NF) for *E. coli* _____

d. Report as *E. coli* Not Found (NF) in 100 mL sample: <1/100 mL _____

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 _____

   c. Report as Total Coliform Present in 100 mL sample: ≥ 1/100 mL _____

3. Place red or magenta containers under Long-Wavelength UV light _____

   a. If the container fluoresces, record test result as Positive (POS) for *E. coli* _____

   b. Report as *E. coli* Present in 100 mL sample: ≥ 1/100 mL _____

   c. If container does not fluoresce, record test result as Not Found (NF) for *E. coli*. Report as < 1/100mL for *E. coli* _____

   d. Report as *E. coli* Not Found (NF) in 100 mL sample: < 1/100 mL _____

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

34. Materials _____

a. Sterile non-fluorescent borosilicate glass or clear plastic tubes 10 mL or 20 mL capacity _____

b. See item 31.b (comparator solution must be in container of same size and type (34.a.) _____

c. See item 31.e _____

35. 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 shaking 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 35.b) 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 _____

l. Examine tubes that exhibit color change for fluorescence _____

36. 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) for Total Coliform _____

4. Record test result of tubes with yellow color equal to or greater than color comparator tube as Positive (POS) for Total Coliform _____

5. Place yellow containers under Long-Wavelength UV light (item 10) _____

a. If the container fluoresces, record test result as Positive (POS) for *E. coli* _____

b. If container does not fluoresce, record test result as Not Found (NF) for *E. coli* _____

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) for Total Coliform _____

3. Record test result of tubes with red or magenta color as Positive (POS) for Total Coliform _____

4. Place red or magenta containers under Long-Wavelength UV light (item 10) _____

   a. If the container fluoresces, record test result as Positive (POS) for *E. coli* _____

   b. If container does not fluoresce, record test result as Not Found (NF) for *E. coli* _____

37. Reporting _____

a. If all tubes exhibit no color change (36.a.3 or 36.b.2), report as Not Found (NF): < 1.1/100 mL for Total Coliform and *E. coli* _____

b. If one or more tubes exhibit color change (36.a.4 or 36.b.3), report as Positive (POS): ≥ 1.1/100 mL for Total Coliform _____

c. If one or more tubes exhibit fluorescence, report as Positive (POS): ≥ 1.1/100 mL for *E. coli* _____

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

38. Materials _____

a. E*Colite substrate, see CP item 27.v _____

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

39. 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 min _____

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 _____

40. Interpretation and Reporting _____

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 _____

c. Place blue or blue/green containers under Long-Wavelength UV light (item 10) _____

1. If the container fluoresces, record test result as Positive (POS) for *E. coli* _____

2. Report as *E. coli* Present in 100 mL sample: ≥ 1/100 mL _____

3. If container does not fluoresce, record test result as Not Found (NF) for *E. coli*. Report as < 1/100mL for *E. coli* _____

MISCELLANEOUS

41. Copy of current in-use edition of <u>Standard Methods for the Examination of Water and Wastewater</u> in laboratory _____