IDEXX
Literature Cover Sheet

IDEXX Library #: 5Z

Topic: US FDA Approval of Colilert for Dairy Source Waters

Title: Milk Laboratory Evaluation Form for Dairy Waters (Coliform Group)

Author(s): US Food and Drug Administration

Date: February, 1994

Source: Form FDA 2400K

Highlights:
• The Chromogenic Substrate (MMO-MUG) Test (Colilert) is approved as either a presence/absence or multiple tube method for testing dairy source waters.
**DAIRY WATERS**  
*(Caliform Group)*

### SAMPLES

1. Laboratory Requirements  
   a. Standard Plate Count Procedures, see items 1-3, (as applicable)  
   b. Sample volume 105±2.5 mL with sufficient air space for mixing (about ¾ full), if completely filled discard  
   c. Transit time does not exceed 30 hours  
   d. If samples are not refrigerated, transit not to exceed 6 hours  
   e. Transported and maintained at 0-4.4°C (temperature control (TC) required)  
   f. Samples examined within 30 hours of collection, or within 2 hours of receipt (item 1d)

### APPARATUS

2. Cultural Procedures, see items 1 - 32 (as applicable)  
3. Sample Containers  
   a. Borosilicate glass, plastic bottles or bags  
   b. Secure, 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)  
5. Fermentation Tubes  
   a. Sufficient size to conform with requirements for media, Durham tube (media must completely cover) and sample  
6. Incubation 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, 2.0 to 3.0 mm in diameter and a minimum of 2.6 cm longer than the fermentation tubes  
   c. Incubating needles  
7. Vacuum source with trap  
8. Membrane filter funnel  
   a. Free from defects which may interfere with function  
   b. Sterilizable  
   c. Mark at 100 mL, or pre-marked, check with Class A graduate cylinder, records maintained  
9. Membrane filters, 47 mm, 0.45 μm (±0.02 μm), sterilized  
   a. Brand  
   b. Lot #  
10. Absorbent pads, sterilized  
11. Forceps  
   a. Round tipped, with smooth surface

### CULTURE MEDIA

12. Culture (Petri) dishes  
   a. Sterile with plastic, light fitting covers  
13. Microscope and Lamp  
   a. Binocular, wide field, 10x oculars  
   b. Fluorescent light, adjacent, above, perpendicular to filter plane  
   c. Other optical device giving equivalent results  
14. Lauryl Tryptose Broth (LST)  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (6.8±0.2)  
15. Brilliant Green Lactose Bile Broth (BGLB)  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (7.4±0.2)  
16. MMO-MUG Medium  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (7.4±0.2)  
17. M-Endo Agar  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (7.4±0.2)  
18. M-Endo Broth  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (7.4±0.2)  
19. Eosin Methylene Blue Agar (EMB)  
   a. Correct composition and preparation  
   b. Brand  
   c. pH (7.1±0.2)  
20. Storage of media  
   a. See Cultural Procedures item 25  
   b. MF Media
TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP
BY MULTIPLE-TUBE FERMENTATION TECHNIQUE

21. Presumptive Test
   a. Lactose Broth or M/M-MUG Medium
      1. Before planting portions arrange tubes in order
         identity samples or otherwise identity
      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. Incubate ten (10) fermentation tubes with 10 mL
         of sample, or five (5) tubes with 20 mL
      5. Incubate at 35±0.5°C for 24±2 hours
      6. Examine tubes for gas - any gas is considered
         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 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 negative for the Test
     12. Do not report gas production after 61 hr of
         incubation
     13. Promptly submit all presumptive positive tubes
         showing gas production at 24 or 48 hr to the
         Confirmed Test

22. 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 hr
      4. Examine tubes for gas - any gas is considered
         positive
      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 negative for the Test
    10. Do not report gas production after 61 hr of
        incubation

23. Reporting
   a. Results of fermentation tubes confirm as positive are
      reported as MPN/100 mL
   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: Negative is <1.1 and Positive is ≥1.1

TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP
BY MEMBRANE FILTRATION TECHNIQUE

24. Filtration
   a. Place (with alcohol lamined forceps) sterile membrane
      filter (item 9) on porous plate, secure funnel
   b. Pour 100 mL test sample into funnel 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 lamined) forceps
   e. M-endo Broth
      1. Sterile pad (item 10) placed in culture dish
      2. Saturate pad with 2.0 mL of M-endo Broth
   f. M-endo Agar
      1. Use culture dish previously prepared (item 20)
      2. Prepared filter placed on agar with rolling
         motion

25. Incubation
   a. In saturated humidity, with dish inverted
   b. At 35±0.5°C for 24±2 hr

26. Counting
   a. Count all dark red colonies, with typical coliforms
      or without (atypical coliforms) sheen and maintain
      separately until confirmed
   b. Confirm 10% up to 10 of colonies, with representative
      proportions of typical and atypical colonised

27. Confirmation Test
   a. Transfer colonies to individual LST and then to BGLB
      tubes using the same transfer needle
   b. Incubate tubes at 35±0.5°C for 24±2 hr
   c. Examine tubes for gas - any gas is considered
      positive
   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 by 24 or 48 hr is considered
      positive for the Confirmed Test
   h. No gas after 48 hr is negative for the Test
   i. Do not report gas production after 61 hr of
      incubation

28. Reporting
   a. Report confirmed colony count/100 mL
   b. Invalidate all samples with confluent growth or NTNC,
      and request a re-sample from the same point source for
      heterotrophic plate count
   c. Interpretation: Negative is <1/100 mL and Positive is
      ≥1/100 mL
### Heterotrophic Bacteria
#### Standard Plate Count Method

**29. Heterotrophic Plate Count Method**

- **a.** Plate samples as in the Standard Plate Count Procedure, items 3-9, 12 and 13
- **b.** Incubate at 35±0.5°C for 48±3 hours
- **c.** Count as in SPC items 15
- **d.** Report counts as in SPC items 16 and 19
- **e.** Record as "Heterotrophic Plate Count/mL at 35°C"

**f. Interpretation:** Negative is <500 CFU/mL and Positive

### Chromogenic Substrate (MMO-MUG)
#### Presence - Absence Screening Test for Dairy Waters (Source Water Supplies Only)

**30. Materials**

- **a.** Color comparator
- **b.** Sterile borosilicate glass or clear plastic bottles to contain 100 mL sample with sufficient air space for mixing (about 1/4 full)
- **c.** Commercially prepared formulation of MMO-MUG substrate
- **d.** Quality control procedures conducted on each lot of substrate received, as recommended by manufacturer, records maintained

**31. Screening 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
- **d.** Incubate at 35±0.5°C for a minimum of 24 hours, not to exceed 28 hours
- **e.** Examine containers for the production of yellow color

**32. Interpretation**

- **a.** If no yellow color is observed
  - **1.** The sample may be recorded as negative for total coliforms
  - **2.** Report as total coliform absent 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 presumptively positive for total coliforms (new sample required for MPN or MF total coliform determination)

### Chromogenic Substrate (MMO-MUG) Multiple Tube Procedure
#### For the Presence of Total Coliforms (Source Water Supplies Only)

**33. Materials,** see items 30 a-d

**34. 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
- **f.** Remove test portions (100 mL total) within 3 minutes
- **g.** Transfer the sample/reagent mixture to five 20 mL, or ten 10 mL tubes
- **h.** Optionally, transfer 100 mL of mbad (see item 34b) sample to MPN tubes containing pre-dispersed MMO-MUG reagent provided by manufacturer
- **i.** Incubate tubes at 35±0.5°C for a minimum of 24 hours, 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

**35. Reporting**

- **a.** If all tubes show no color, report as negative: <1.1/100 mL
- **b.** If one or more tubes show yellow color (see 34j) report as positive: MPN/100 mL

### Miscellaneous

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

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Form FDA 2400k (Rev. 2/94)