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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
MILK LABORATORY EVALUATION FORM

LABORATORY

LOCATION | LAB #

EVALUATION BY: | DATE | X = DEVIATION O = NOT USED | U = UNDETERMINED NA = NOT APPLICABLE

DAIRY WATERS
(Coliform 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 0-4.4C (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. 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 _____) .....
5. Fermentation Tubes .....
a. Sufficient size to conform with requirements for media, durham tube (media must completely cover) 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, 2.0 to 3.0 mm 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 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 .....
Brand _____ Lot # _____ .....
10. Absorbent pads, sterilized .....
11. Forceps .....
a. Round tipped, with smooth surface .....
12. Culture (Petri) dishes .....
Brand _____ Size _____ .....
a. Sterile with plastic, tight fitting covers .....
13. Microscope and Lamp .....
Make _____ 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. Lauryl Tryptose Broth (LST) .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (6.8±0.2) .....
15. Brilliant Green Lactose Bile Broth (BGLB) .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (7.4±0.2) .....
16. MMO-MUG Medium .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (7.4±0.2) .....
17. M-Endo Agar .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (7.2±0.2) .....
18. M-Endo Broth .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (7.2±0.1) .....
19. Eosin Methylene Blue Agar (EMB) .....
a. Correct composition and preparation .....
b. Brand _____ Lot No _____ .....
Rcd Date _____ Date Opened _____ .....
c. pH _____ (7.1±0.2) .....
20. Storage of media .....
a. See Cultural Procedures item 25 .....
b. MF Media .....
1. Store in dark at 0-4.4C .....
2. Broth medium used within 96 hr .....
3. Plates kept no more than 1 week in a sealed container at 0-4.4C .....

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

21. Presumptive Test .....
a. Lauryl Tryptose Broth or MMO-MUG Medium .....
1. Before planting portions arrange tubes in order identify samples 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 .....
5. Incubate tubes at 35±0.5C 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 51 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±0.5C 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 51 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 flamed 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 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 Broth, item 18 .....
3. Allow to stand a few minutes before pouring off excess .....
4. Prepared filter rolled (grid side up) onto pad .....
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±0.5C for 23±1 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 colonies .....
27. Confirmation Test .....
a. Transfer colonies to individual LST and then to BGLB tubes using the same transfer needle .....
b. Incubate tubes at 35±0.5C 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 51 hr of incubation .....
28. 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: Negative is <1/100 mL and Positive is ≥1/100 mL .....

LABORATORY	LAB #	LOCATION	DATE

DAIRY WATER (Continued)

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±0.5C for 48±3 hours .....
c. Count as in SPC item 15 .....
d. Report counts as in SPC items 16 and 19 .....
e. Record as "Heterotrophic Plate Count/mL at 35C" .....
f. Interpretation: Negative is <500 CFU/mL and Positive is ≥500 CFU/mL. .....

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

30. Materials .....
a. Color comparitor .....
b. Sterile borosilicate glass or clear plastic bottles to contain 100 mL sample with sufficient air space for mixing (about ¼ 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±0.5C 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 comparitor dispensed into the SAME type of sample container .....
3. If color is equal to or greater than that of the color comparitor, 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 mixed (see item 34b) sample to MPN tubes containing pre-dispensed MMO-MUG reagent provided by manufacturer .....
i. Incubate tubes at 35±0.5C for a minimum o 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 comparitor tube (SAME size and type as MPN tubes) .....
3. Tubes with color equal to or greater than color comparitor 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 .....