IDEXX
Literature Cover Sheet

IDEXX #: 9B

Title: The Fecal Coliform Test Compared To Specific Tests For *Escherichia coli*

Topic: Fecal Coliform Vs *E. coli*

Source: IDEXX

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Highlights:

- *E. coli* is the only member of the coliform group that unquestionably is an inhabitant of the intestinal tract and it has become the definitive organism for demonstrating fecal pollution of water.
- Colilert (Minimal Media ONPG-MUG Test; MMO-MUG) is a direct test of water samples and offers the advantage of simultaneous determination of both total coliforms and *E.coli* within 24 hours, without the need for confirmatory testing. Setup is simple and results are clearly and easily read with the aid of a comparator.
- The Fecal Coliform test picks up thermotolerent coliforms other than *E.*coli. Estimates in the literature are that 15% of positive thermotolerant Fecal Coliform test results are due to coliforms other than *E. coli*. Many of these results are due to *Klebseilla* isolates that are ubiquitous in the environment, not of fecal origin and not connected to the occurrence of human disease. False negative results due to non-gas producing strains of *E.coli* have been reported to approach 10% of the *E. coli* population.
- The EC Medium plus MUG test, is a separate test that sequentially follows presumptive and confirmatory coliform tests of the original water sample. It requires a separate incubator or waterbath rigidly controlled at 44.5°C ± 0.2°C. The MUG reactions in EC medium plus MUG are not as clearly read as Colilert, requiring various positives and negative controls.

* See underlined and highlighted areas

The Fecal Coliform Test Compared To Specific Tests For *Escherichia coli*

1. Indicator Organisms for Fecal Contamination: History, and the Importance of *Escherichia coli*

1.A. Total Coliforms and *E. coli*

As reviewed by Pipes (1), Cliver, Newman and Cortruvo (2), and others, the indicator organism for fecal contamination of drinking water was originally specified as *Bacillus coli* (now *Escherichia coli*), the organism found in feces in large numbers which fermented lactose with the production of acid and gas. Early investigators recognized the difficulties in isolating pathogens compared to the relative ease in isolating *B. coli* from polluted waters. Because *B. coli* was regularly associated with feces, was present in water in numbers greater than those of pathogens, and survived longer in water than pathogens, B. coli was established as the indicator of the sanitary quality of water. Therefore, *B. coli* was recommended by the American Public Health Association as the bacteriological indicator for water in the first edition (1905) of Standard Methods of Water Analysis. Similarly, the first bacteriological quality standards for potable water in the U.S. issued in 1914 specified *B. coli* as did subsequent regulations issued in 1925.

It was later realized that there were several species of bacteria belonging to various genera that produced gas from lactose, and these *B. coli*-like bacteria became known as the "coliform group" of bacteria. Largely because of lack of simple methods to separate members of this "coliform group", coliforms were used as indicators in place of *B. coli* in later regulations issued in 1943. The "coliform group" has continued to be used as an indicator, but in recent years serious questions about their use have arisen. For example:

- Total coliforms can grow in water of low organic matter content and of low temperature, and this ability to multiply readily in the environment make total coliforms of limited value as direct indicators of fecal contamination in raw source waters.

- Total coliforms have been recovered from soil, on vegetation, in forest and farm products, and in various other environments, including those almost untouched by humans.

- Growth of coliforms on the interior surfaces of water mains is a widespread occurrence, and these biofilm coliforms may be shed into the water. These coliforms have not been related to any health effects but their presence in a water distribution system results in violations of the standards (reviewed by Pipes, 1).

1.B. Thermotolerant Coliforms ("Fecal Coliforms")

For many years, the total coliform group served as the main indicator of fecal contamination, but since many of the organisms in this group are not limited to fecal sources, an attempt was made to develop a method to determine those coliforms which were more clearly of fecal origin. *E. coli* was known to be more thermotolerant than non-*E. coli* coliforms (Eijkman, 1904; Zent. Bakteriol., Abth.I.Orig. 37:742-752). A method was devised based on this observation of thermotolerance, the fermentation of lactose with gas production at 44.5°C in EC Medium (Hajna and Perry, 1940; Amer. J. Pub. Health 33:550-556). While aimed

at *E. coli*, the test also detects other thermotolerant coliforms, especially of the genus *Klebsiella*. Since *Klebsiella* isolates may originate from non-fecal sources, many scientists think the term "thermotolerant coliforms" is more accurate and representative than the term "fecal coliforms" for describing positive results from this tests (Cliver, Newman and Cortruvo, 2).

Since *E. coli* is one of the principle species making up the thermotolerant coliform group, the thermotolerant coliform test is valuable for an indication of the potential presence of enteric pathogens in water, but where the means are available, *E. coli* is the preferred indicator because it excludes most of the *Klebsiella* organisms which may or may not originate from fecal sources (review and conclusions of NATO/CCMS Drinking Water Microbiology Committee; Cliver, Newman and Cortruvo, 2).

Since *E. coli* is the only member of the coliform group that unquestionably is an inhabitant of the intestinal tract, it has become the definitive organism for demonstrating fecal pollution of water. *E. coli* meets the criteria of a valid fecal indicator in that: it is present in the intestine in numbers larger than those of enteric pathogens; it behaves similarly to enteric pathogens within the aquatic environment; and it is less susceptible than most enteric pathogens to treatment or disinfection procedures. The presence of *E. coli* in a water supply indicates contamination with fecal material from warm-blooded animals such as birds and humans. One must assume that, if *E. coli* has gained access to a waterway, enteric pathogens also may have entered this water (Cliver, Newman and Cortruvo, 2).

2. Fecal Coliform Test - Thermotolerant Positives other than *E. coli*: False Positives

The Fecal Coliform Test (production of gas from lactose in EC Medium at 44.5°C), viewed as a surrogate test for *E. coli*, can yield a significant percentage of false positive results due to thermotolerant coliforms. Estimates in the literature are that 15% of positive thermotolerant Fecal Coliform test results are due to coliforms other than *E. coli*.

Many of these results are due to *Klebsiella* isolates. *Klebsiella* are ubiquitous in the environment and originate from a wide variety of sources including both vegetable and animal sources (reviewed by Geldrich, 3; Cliver, Newman and Cortruvo, 4). Approximately one third of all warm blooded animals including man have *Klebsiella* in their intestinal tracts. However, the majority of *Klebsiella* encountered in water are environmental strains that originated from vegetation, agricultural products, wood pulp and paper mill effluents and textile industry wastes. *Klebsiella* have been isolated from living wood of redwood, white fir trees and southern pines, sugar cane wastes, kelp processing, cotton, fresh vegetables, and tree needles and bark in a forest environment including virgin forests of British Columbia. Evidence that *Klebsiella* and *Enterobacter* recovered from water stored in redwood storage tanks actually came from the redwood itself was reported(Bagley et al, 5). Of note is the ability of *Klebsiella* to grow in nutrient-rich waters such as pulp and paper mill effluents, sugar refining and processing, etc., so that *Klebsiella* occupies a dominant position among coliforms in many such effluents (Geldreich, 4, Table 2). Numerous references to the non-fecal environmental origins of *Klebsiella* may be found in Attachments 3, 4 and 6.

Not all *Klebsiella* give a positive result in the thermotolerant Fecal Coliform Test; however, most Fecal Coliform Test positive coliforms other than *E. coli* are *Klebsiella* (Dufour, 1977; Bacterial Indicators/Health Hazards Associated with

Water, ASTM STP 635; cited in Attachment 4). Those *Klebsiella* which can grow at 44.5°C and are Fecal Coliform Test positive are not necessarily of fecal origin however. Various studies are cited in the review articles, but the following studies may serve to illustrate this fact.

Caplenas and Kanarek (6) conducted a study of pulp and paper mill processing plants in which concentrations of both non-thermotolerant (Fecal Coliform Test negative) and thermotolerant (Fecal Coliform Test positive) *Klebsiella* were studied. Up to 90% of non-fecal source thermotolerant *K. pneumoniae* were falsely identified as fecal source bacteria. Citing their findings, and other studies that also found high densities of fecal coliform bacteria in waters that did not receive human or animal wastes, the authors concluded that the thermotolerant Fecal Coliform Test lacks the specificity required for determination of fecal contamination standards, and called for a more reliable health risk assessment of fecal contamination based on *E. coli*. The tremendous regrowth potential of these non-fecal source *Klebsiella* was also pointed out. An indicator organism should not be capable of multiplication once in the water.

A second study investigated possible health risks associated with elevated total coliform counts in a distribution system of a public water supply serving 350,000 people (Edberg et al, 7). As part of the study they compared bacterial isolates from the distribution system to isolates of the same species obtained from a large regional hospital and from a national compendium of clinical isolates. Temperature tolerance at 44.5°C in EC Medium (Fecal Coliform Test) was one of the characteristics analyzed. A total of 80% of *Klebsiella pneumoniae* and 50% of the closely related *K. oxytoca* from the distribution system were positive in the thermotolerance Fecal Coliform Test, as were 10 of 37 *Enterobacter cloacae* isolates and 2 of 8 *E. agglomerans* isolates. Since other evidence gathered in the study had suggested the *Klebsiella* and *Enterobacter* isolates were of environmental origin, not fecal origin, the evidence suggested that the thermotolerance Fecal Coliform Test may not conclusively identify either *E. coli* or the fecal origin of other coliforms. The authors cited other studies in which significant percentages of coliforms other than *E. coli*, notably *Klebsiella* species, had demonstrated positive Fecal Coliform Tests despite the original premise of the test, namely, that *E. coli* will grow and metabolize at 44.5°C while other Enterobacteriaceae will not.

An EPA study which assessed the role of indicator organisms for the quality of recreational waters (8) noted that fecal coliforms had been faulted because of the non-fecal sources of at least one member of the fecal coliform group, *Klebsiella*. Studies were cited in which thermotolerant *Klebsiella* were observed from various sources free of fecal contamination, including pulp and paper mill effluents (J. Water Poll. Control Fed., 1976, 48:1776; Appl. Environ. Microbiology, 1981, 42:779), textile processing plant effluents (J. Water Poll. Control Fed., 1976, 48:872), cotton mill wastewaters (Can. J. Microbiol., 1976, 22:1762) and sugar beet wastes (Appl. Microbiol., 1968, 16:1875). The same EPA study (8) concluded that the previously recommended indicator organism group for recreational waters, the fecal coliforms, was inadequate. The freshwater studies showed that enterococci and *E. coli* had equally strong correlation with swimming associated gastrointestinal illness, but fecal coliforms showed poor correlation. The EPA urged that all waters that were classified for primary contact would benefit from application of the revised and updated criteria based on enterococci and *E. coli*, rather than fecal coliforms.

Since *Klebsiella* is the most common non-*E. coli* thermotolerant Fecal Coliform, a judgment as to its validity as an indicator of fecal contamination of water and its overall importance in water is required. The NATO/CCMS Drinking Water Microbiology Committee (Cliver, Newman and Cortruvo, Attachment 4) has reviewed *Klebsiella.* They noted its widespread presence in the environment. They noted that although it was carried by many healthy individuals, *Klebsiella* could be an opportunistic pathogen. Also, in some studies, strains from environmental sources could not be readily distinguished from clinical isolates when tested biochemically, serologically, or by thermotolerance. However, they concluded that there was no epidemiological evidence to connect the incidence of *Klebsiella* in drinking water or recreational waters with the occurrence of human disease. This conclusion was also reached in a review by Duncan (9).

The NATO/CMS Drinking Water Microbiology Committee (Attachment 4) also concluded that *Klebsiella* cannot be considered reliable indicators of fecal pollution because

1) they are not always present, or found in high numbers in feces
2) they are found in large numbers in certain industrial wastes
3) they are ubiquitous in the environment
4) they are able to multiply in nutrient-rich waters.

3. Fecal Coliform Test - False Negatives

Although the thermotolerance Fecal Coliform Test will produce positive results with some coliforms other than *E. coli*, the meaning and value of these non-*E. coli* positives as they pertain to fecal contamination is, then, uncertain. Of equal or greater importance is a consideration of what is not found by the Fecal Coliform Test, which is based on formation of gas from lactose at elevated temperature, 44.5°C.

Anaerogenic (non-gas producing) strains of coliforms will produce false negative reactions (no-gas produced, but coliforms and even thermotolerant coliforms are present) in the presumptive (LTB), confirmed (BGB Broth) and fecal coliform stages of testing in MPN or Presence-Absence Broth Tests; and in the confirmation (LTB and BGB Broth) and fecal coliform stages of membrane filtration. An anaerogenic thermotolerant (and therefore, potentially fecal) coliform will be missed entirely, even as a total coliform, by conventional methods based on production of gas from lactose in broth media.

Especially important because of the definitive role of *E. coli* as an indicator of fecal contamination, anaerogenic isolates of *E. coli* would not be detected by conventional methods based on gas production. Non-gas producing strains of *E. coli* have been reported to approach 10% of the *E. coli* population (10,11). The Alkalescens-Dispar group contains *E. coli* strains that typically are anaerogenic (12,13).

The public health implications of failing to detect contaminated water because of anaerogenic coliforms led to a study of alternative verification methods that were independent of gas production (14). Of 682 presumptive coliforms cultures from 21 contaminated drinking and surface water samples, 84.6% were verified as coliforms by a beta-galactosidase/cytochrome oxidase enzyme determination that did not rely on gas production. Using conventional methods, only 58.9% of the 682 presumptive coliforms were verified as coliforms in LTB broth by gas production, and only 50.4% were verified in BGLB broth by gas production.

Identification of the anaerogenic lactose-fermenting coliforms (LTB) that were verified by the enzyme method revealed that 91.5% belonged to one of the four commonly accepted coliform genera; *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter*. Of special note was that *E. coli* was the second most common anaerogenic coliform at 23.9%. The authors concluded that verification of presumptive coliform colonies from membrane filters using standard procedures which depend on gas production may result in significant underestimation of indicator organisms. The authors also cited other factors that could severely influence the sensitivity of the membrane filter procedure, including elevated turbidity, injured coliforms, high numbers of noncoliform bacteria (heterotrophic interference rule) and membrane filters.

Another study showed that variation in gas production by *E. coli* is not due only to differences among *E. coli* strains; multiple subcultures from individual strains also exhibited variable gas production (15). False negative reactions (growth of *E. coli* without gas) have usually been interpreted as chance cultivation of anaerogenic or environmentally damaged strains, but this study showed that this need not be the case since variability arose even among subcultures of known gas-producing strains of *E. coli*..

A study of 240 *E. coli* cultures originally isolated from a variety of water samples showed that 11.7% examined with the standard Fecal Coliform Test failed to produce a positive response based on gas production in EC Medium at 44.5°C (16). All 28 of these EC Medium gas negative *E. coli* cultures were MUG positive in the Colilert test. Only 1 of the 28 was MUG negative in EC Medium plus MUG at 44.5°C. Only 12 of the *E. coli* cultures were judged to be true anaerogenic strains since they did not produce gas in any conventional lactose fermentation medium at 35 or 44.5°C. Ten of these isolates were detected by MUG reactions of EC plus MUG and Colilert, and all 12 were positive for ONPG in Colilert.

4. Heterotrophic Interference

The Fecal Coliform Test using EC Medium, and the EC Medium plus MUG test for *E. coli* are tests which follow, in sequence, the accurate running and reading of the presumptive and confirmatory stages of coliform testing. Suppression of *E. coli* and total coliforms in LTB or on membrane filters grown on m-Endo media can result in false negative presumptive tests for coliforms, and therefore, for *E. coli* or fecal coliforms. This interference by non-coliform heterotrophs has resulted in the heterotrophic interference rule which requires invalidation of the test sample if: a) there is a turbid broth culture in the absence of gas production (or acid) using an analytical method where gas formation (or acid) is examined b) membrane filter exhibits confluent growth or produces colonies too numerous to count, without coliform colonies seen (Federal Register, 6/29/89, Vol. 54, pages 27544-27568; 17). In the required retesting of such samples, the EPA recommended using an analytical method that is less vulnerable to interference by high levels of heterotrophic bacteria, namely Colilert. National field trials have shown Colilert to be unaffected by high heterotrophic levels (18, 19).

5. Recovery of Chlorine - Stressed *E. coli*

EC Medium plus MUG was based on the historical acceptance of EC Medium as used for fecal coliforms, with the advantage of specificity for *E. coli*. Of importance then, was the demonstration that Colilert was also capable of detecting

chlorine-stressed *E. coli*. In studies conducted by the EPA (Covert et al, 20), or designed and reviewed by the EPA and the American Water Works Association (Standridge et al, 21), Colilert was shown to recover low densities of stressed *E. coli* satisfactorily, being equal to or superior to EC Medium plus MUG (Federal Register, Attachments 22, 23).

6. Test Procedures

The Fecal Coliform Test for thermotolerant coliforms (EC Medium at 44.5°C), and the EC Medium plus MUG test for *E. coli* (44.5°C), are each separate tests that sequentially follow prior presumptive and confirmatory coliform tests of the original water sample. Final results may take days. In contrast, total coliform and *E. coli* testing is performed simultaneously on the same water sample with Colilert, giving results within 24 hours.

In addition to the standard 35°C coliform incubator, the Fecal Coliform Test and the EC Medium plus MUG test require a separate incubator or water bath rigidly controlled at 44.5°C ± 0.2°C. It has been shown that decreases as little as 0.2°C below 44 will permit a much higher percentage of the non-fecal Klebsiella to yield a positive test and temperatures as little as 0.2°C above 45 will inhibit the growth of many strains of *E. coli* (24). With Colilert, total coliform and *E. coli* testing is conducted simultaneously at 35°C, requiring only one incubator temperature.

Over-inoculation of Fecal Coliform tests, which is very difficult to control, has been reported to significantly increase the number of fecal coliforms (24).

USEPA Test Method No. 1104, "Detection of *Escherichia coli* in Drinking Water with Mug Tube Procedure" (25) cautions about certain interferences and difficulties of the test:

- certain brands of test tubes fluoresce under long-wave UV light and may interfere with test results. Tubes should be examined before use.

- certain lots of EC plus MUG media may auto fluoresce. Each lot of medium should be checked before use with the UV light to insure that it does not fluoresce. To insure that weak auto fluorescence of the medium, if present, is not misinterpreted as positive for *E. coli*, a MUG-positive (*E. coli*) and MUG-negative (uninoculated) control are necessary for each analysis.

- an inverted vial for gas determination is not allowed. Gas production is not relevant to the test and observation for this reaction may cause confusion in test interpretation.

- verification is required for at least 5% of both MUG-positive results and turbid MUG-negative results.

Additional difficulties in reading the EC Medium plus MUG test were noted in EPA studies. Covert et al (20) noted that a positive MUG test using Colilert was easy to detect with brilliantly fluorescing tubes; however, the MUG reaction was sometimes difficult to interpret with EC plus MUG tubes that showed heavy growth. Shadix and Rice (16) also found the turbidity due to heavy bacterial growth in lactose-based MUG media (EC plus MUG) often made reading the fluorescence of the MUG reaction difficult. MUG positive (*E. coli*) and MUG-

negative (*Klebsiella pneumoniae*) controls were needed for comparison. Therefore, in addition to the required uninoculated and MUG-positive (*E. coli*) controls, a turbid MUG-negative control (thermotolerant *Klebsiella*) is recommended. In contrast, they found that the MUG reaction was easier to read in the Colilert tests.

A comparator is provided for judging Colilert results. This is a solution, prepared by the manufacturer, that exhibits the minimum intensity of a positive Colilert reaction. A comparator does not exist for judging MUG reactions in EC Medium plus MUG.

7. Summary

For sometime, *Escherichia coli* has generally been considered to be a definitive indicator of fecal contamination in drinking water. It is routinely found in large numbers in the intestine of warm blooded animals including man, and does not persist for long in the environment as a free living organism. Therefore, when found in environmental samples, *E. coli* represents evidence of recent fecal contamination.

In contrast, coliform bacteria other than *E. coli* may be found in the intestine of warm blooded animals, but may also be found free-living in the environment. Therefore, their detection in environmental samples such as water does not constitute definitive evidence of fecal contamination of the sample, as does *E. coli*.

Until recently, uncomplicated direct methods for the specific detection and enumeration of *E. coli* in water samples, usable by all laboratories involved in such testing, did not exist. Instead, a surrogate test aimed at the thermotolerance of *E. coli* was used as a measure of the possible fecal origin of coliforms encountered in water samples. The test is based on the fermentation of lactose, complete to the production of gas, in EC Medium at the elevated temperature of 44.5°C. Although aimed at *E. coli*, coliforms other than *E. coli* could give positive reactions, and the test could not detect non-gas producing strains of *E. coli*. Since up to 10% of *E. coli* may not produce gas (anaerogenic), this represents a gap in specific detection of fecal contamination. Positive reactions by thermotolerant coliforms other than *E. coli* do not add a counteracting safety margin since these thermotolerant coliforms may not be of fecal origin.

Currently, specific tests for *E. coli* have been developed, and have received approval by the USEPA for use in testing of drinking water. Colilert (Minimal Medium ONPG-MUG Test; MMO-MUG) and EC Medium plus MUG, each based on the beta-glucuronidase enzyme which is specific to *E. coli* among the coliforms, offer to all laboratories simple tests for specific detection of fecal contamination, *E. coli*: Colilert is a direct test of the water sample and offers the advantage of simultaneous determination of both total coliforms and *E. coli* within 24 hours, without the need for confirmatory testing. Setup is simple and results are clearly read.

In contrast, EC Medium plus MUG is a separate test that follows, in sequence, the accurate running and reading of the presumptive and confirmatory stages of coliform testing. These earlier stages of standard testing are subject to interference by non-coliform heterotrophs that requires invalidation of the test sample. In the required retesting of such samples, the EPA recommended using

an analytical method that was less vulnerable to interference by high levels of heterotrophic bacteria, namely, Colilert.

In studies conducted by or designed by the USEPA, Colilert has been shown to be as good or better than EC Medium plus MUG for recovering chlorine-stressed *E. coli.*

The MUG reaction in Colilert is easily read, aided by a Comparator to judge threshold intensity of positive reactions, does not require additional incubator temperatures other than that used for total coliforms, and provides results on *E. coli* within 24 hours of sample setup. MUG reactions in EC Medium plus MUG are not as clearly read, requiring various positive and negative controls for each test run, additional 44.5°C ± 0.2°C incubation is needed, and the overall sequence of testing may require days till there is a judgment on fecal contamination.

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