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Comparison of Standard Methods, MTF, and MF, by the Colilert Rapid test for the Enumeration of Coliform Bacteria in High Salinity Water, Arabian gulf, Doha, Qatar

Magda M. Abed El-Atty EL-Magharaby*

Abstract: Colilert Rapid test was compared with the traditional methods (multiple tube fermentation (MTF) and Membrane filtration (MF)) to enumerate total and fecal coliform in marine water. Colilert method is based on specific indicator nutrients: ONPG and MUG for the target microbes, and chemically suppresses non-coliform bacterial growth. It is more rapid, providing results in as little as 18 h compared to the 24–96 h required for traditional methods. The results reported that Colilert method was more sensitive in detecting fecal coliform than the traditional methods (MTF and MF). No significance difference between it and MF in enumeration of total coliform. While, there were significant differences with both MF and MTF in enumeration of fecal coliform. It was more close to MF than MTF. The study recommended MF technique for detecting and enumerating coliform bacteria in marine water due to the high values of false positive results detected by Colilert method.

INTRODUCTION

The coastal water is an important economic and recreational resource that is influenced by human activities.(1) The hygienic quality of water is of utmost importance to society, and efficient bacteriological control of water is essential for implementing a good management of this vital resource.(2) The main criterion for assessing the potential health risk of waters is the density of indicator bacteria. Although indicator bacteria do not necessarily cause illness, they are abundant in human waste where pathogenic organisms, such as pathogenic bacteria, viruses, and parasites are also likely to exist.(3) The typical indicators used include total coliform, fecal coliform, *Esherichia coli*, and enterococci.(4) Total and fecal coliform were recommended by the US Environmental Protection Agency (EPA) in 1976.(5) Indicator bacteria have historically been measured using either membrane filtration (MF) or multiple tube fermentation (MTF), which both as a rule require 24 to 48 h for completion(6).The

* Environmental Health Department, Environmental Chemistry and Biology, HIPH.

Colilert reagent, based on IDEXX's defined substrate technology, is used for the simultaneous detection and confirmations of total coliforms and *E. coli* in water. It provides specific indicator nutrients: ONPG (O-Nitrophyenyl-B-D-Galactopyran-oside) and MUG (4-Methylum-belliferyl-B-D Hlucuronide) for the target microbes, and chemically suppresses non-coliform bacterial growth. This reagent system is specifically formulated to achieve optimum sensitivity and specificity in the simultaneous detection and identification of total coliform and ***E. coli***. After 24 hours incubation at 35°C, if ***E. coli*** is present, the reagent should show yellow color and fluorescence when exposed to a long-wave (365-366 nm) UV lamp.[7] The allure of the Colilert test is that it is more rapid, providing results in as little as 18 h compared to the 24–96 h required for traditional methods. It has the additional advantage of being less expensive [3].Qatar is located half way along the western coast of Arabian Gulf. The total area of Qatar's sea waters are approximately 35,000 sq. Km (approximately 15% of the Gulf). Qatar has an extremely shallow sea with an average depth of 30 m. The high evaporation of the water especially in summer, the very low rainfall, and the little fresh water inflow from the land are the main factors which produce very high sea water salinity. The salinity within Qatar's waters varies mainly between 39 ppt and 41 ppt at the surface. At the bottom, close to the offshore boundaries salinity tends to be 1 - 2 ppt higher than that at the surface.[8] So, two major objectives of the present study: are to compare the Colilert method with the standard MTF and MF in quantification of coliform bacteria, and to assess the efficient one used in high salinity water.

MATERIAL AND METHODS

Sea water samples, (n=32), were collected randomly from the Doha coastal region. Samples were collected aseptically

in 250 mℓ sterile Pyrex glass bottles, placed on ice and transported immediately to the laboratory and analyzed within an hour. MTF was carried out by using lactose broth for presumptive test and Brilliant green bile broth for confirmed test to estimate the total coliform density and Eijkman medium at 44.5°C to estimate the fecal coliform density. MF total Coliform and fecal coliform analyses with m-Endo Agar LES (Fluka) and mFC Agar (Fluka) media respectively were used to estimate the total coliform and fecal coliform densities. The media used and analytical methods were performed in accordance with the standard procedures outlined in standard methods [6]. The estimation of total coliform and *E. coli* by Colilert test was performed in accordance with Standard Operating Procedure for: *Escherichia coli* and Total Coliform using the IDEXX Quanti-Tray/2000 System with Colilert reagent (4010R01 *E. coli* IDEXX.doc).[9] The last ten samples were performed to intensive tests to recovery of coliforms and *E. coli*.

Recovery of coliforms. Following incubation, the backing material of each Quantitray was disinfected by application of 70% ethanol with a sterile swab. After the residual ethanol evaporated, sterile pipette tips were used to pierce the backing material of all MUG-positive, ONPG-positive wells; all MUG-positive, ONPG-negative wells; all part florescent, two ONPG-positive wells and MUG-negative per tray. One tray was processed per each water sample. One hundred micro-liters of fluid were withdrawn from each well and added to a separate tube containing 5 ml of Ej broth with a Durham tube and incubated at 44.5°C. After 24 h, all of the tubes were examined for gas formation. At the same time the Ej tubes were inoculated, fluid from each well was used to inoculate selective-differential media. One drop (approximately 20 µl) of well content was

streaked for isolation on MacConkey agar and EMB agar. Following incubation at 35.0°C for 24 h, colonies were examined for lactose utilization. Selected colonies isolated from each Colilert well were stained with gram stain and cultured on tryptone water and incubated at 35°C. After 48h, all were examined for indol formation. From the positive MTF fecal coliform tubes, full loop from each was streaked also on MacConkey and EMB agar and then stained and tested for indol formation. Selected one typical blue colony (fecal) from positive m-FC agar were stained and cultured on tryptone water and incubated at 35°C. After 48h, all were examined for indol formation.

Statistics:

Descriptive analysis, AOVA test , LSD, and Sensitivity tests were done on the results.

RESULTS AND DISCUSSION

Total coliform

Results revealed that 12.5% from samples undetected of total coliform by MTF and 9% by MF while no samples undetected for total coliform by the Colilert method. MTF method recorded the highest value of total coliform in 9.4% of samples, MF method recorded the highest value in 37.5% of samples , and Colilert method recorded the highest value in 53.1% of samples.

Two samples (6.25%) were coliform negative by MTF and MF procedure but coliform positive by the Colilert method. Covert *et al*.,[10] reported one sample only was coliform negative by MTF and MF procedure but coliform positive by the Colilert method.

For the MTF method, the geometric mean value of total coliform was 1.8 MPN/100 ml with standard error of 0.290 MPN/100 ml. For the MF, the geometric mean value was 2.9 CFU/100 ml with standard error of 0.192 CFU/100 ml .For the Colilert method, the geometric mean value was 3.3 MPN/100 ml with standard

error of 0.133 MPN/100 ml as presented in table (1).

A number of environmental factors injure or kill coliform bacteria in seawater.[11] Furthermore, salinity has been shown to be detrimental to the survival of *E. coli* and other coliform bacteria, with aged seawater being more toxic than fresh water.[12] The lower recovery efficiencies of coliform may be due to the inhibition of biochemical reactions producing typical positive results in both MTF and MF assay methods. [13]

Attributed to the MTF, the lower values detected may be due to that the enzyme formic hydrogenlyase (which produces hydrogen gas from formic acid) may be impaired or not induced in environmentally stressed cells.[14] An injury of this nature could explain the absence of gas production in the presumptive portion of the MTF test. This is the reason for giving false negative result and consequently lower count recorded by this method.

Olson[14] stated in his study on seawater, a mean increase of 10.4% in the total coliform count occurred when incorporate of false negative into the coliform MPN method number. Total coliform results by Colilert about 1.8 and 1.1 higher than MTF and MF methods respectively. Noble *et al*.,[15] reported total coliform were 1.6 to 1.8 times by Colilert higher than those for MF and MTF.

It was noticed that the highest geometric mean value of total coliform reported by the Colilert method, this may be indicated that this method was more sensitive than the standard methods or it may give false positive result due to the presence of interference of bacteria other than coliform bacteria. Previous study [16] indicated that various plant and algal extracts can significantly interfere with the Colilert system's detection of both coliform and *E. coli*.

In an earlier study, Palmer *et al*.,[17] also reported higher total coliform recovery in the marine environment when the original

Colilert was used. They hypothesized that the Colilert medium did a better job of resuscitating coliforms stressed by exposure to highly saline bay water than does conventional recovery medium.

Table (2), contributed to tota l coliform detection, 87.5% from samples concordance between Colilert and MTF while 90.6% samples were concordant between Colilert and MF.

The present study reported only 40% true positive coliform as shown in figure (1), when co-cultured from positive Colilert (yellow well) on selective media for coliform bacteria (Mac-conkey agar and EMB agar). Based on this finding, the geometric mean value of total coliform decreased to about 1.3 MPN/100 ml.

Edberg *et al*.,[18] found that 82% of the isolates from positive Colilert were members of total coliform group while Terry *et al*.,[10] reported only 73% members of coliform grouping in drinking water. Carlucci and Parmer[11] reported over 75% of all isolates from marine sites, indicating the presence of the family *Vibironaceae* members (non coliform members). Palmer *et al*.,[17] reported false-positive rates of 19% for total coliform. Published studies suggest that there are substantial false positives, yielding higher total coliform counts from marine water.[17,20]

Fecal coliform

Colilert rapid method detects *E .coli* directly but the standard methods detect fecal coliform as general and then by biochemical tests the *E. coli* can be detected where *E. coli* is a member of fecal coliform.

Result revealed that as shown in figure (2), the three different methods agree of detecting fecal coliform in 3% only from samples in which Colilert detected the highest value (200 MPN/100 ml). 6% from samples detected fecal coliform by both Colilert and MF, figure (2) and table (3), from which 3% detect the higher value by Colilert (300 MPN/100 ml). The same

samples percent detected fecal coliform by both MF and MTF as shown in figure (2), while the highest percent of samples (9%) detected fecal coliform by both Colilert and MTF, figure (2) and table (3), from which6% detect the higher value by Colilert (200 MPN/100 ml).

Attributed to the undetected fecal coliform, figure (3) showed that about 44% from samples undetected fecal coliform by the three methods. The highest percent of undetected, 67%, reported by both MF and MTF. 59% from samples recorded undetected fecal coliform by both Colilert and MTF while both Colilert and MF undetected fecal coliform in 47% from samples.

It was noticed that, 25% from samples detected *E. coli* by Colilert method and undetected fecal coliform by other methods, although *E. coli* is considered as a member of fecal coliform. 12.5% from samples detected fecal coliform by MF and undetected by others while 3% only detected fecal coliform by MTF and undetected by others.

Generally, the Colilert method reported the highest geometric mean and standard error values of 0.8 and 0.184 MPN/100 ml, respectively followed by MTF with geometric mean of 0.3 MPN/100 ml. The lowest mean value (0.2 CFU/100 ml) was recorded by MF method. The MF detected the lowest standard error (0.086) as presented in table (1). Fecal coliform as *E. coli* result by Colilert were 2.7 and 4.0 times higher than those for MTF and MF methods, respectively.

The confirmation of positive *E. coli* , figure (4), detected by Colilert method by streaking on EMB agar, gram staining, and indol test indicated that 33% were false positive which were yellow color and fluorescent but indol negative and 5% was false negative which were yellow color and non-fluorescent but indol positive.

Palmer *et al*.,[17] reported false-positive rates of 15% for *E. coli.* They stated that

not all *E. coli* are MUG positive–false negatives; not all fluorescent organisms are *E. coli* – false positives.

The confirmation tests on the positive fecal coliform, gas production at 44.5°C, by MTF indicated that no *E. coli* was recovered while it was recovered from MF technique (indol +ve and –ve gram stain).

Colilert method produces 12,5% false negative *E. coli* results as stated by Schets *et al*.[21] They reported that, Colilert is considered less suitable for direct detection of *E. coli* because of the occurrence of high percentage false negative results due to the inability of a fraction of the *E. coli* population to use the substrate incorporated in Colilert for *E. coli* detection.

Colilert -24 and Colilert -18 should not be used to test for total coliform and *E. coli* on water-bodies where conductivity exceeds 10,000*u*S.[7]

Qatar marine water has high conductivity which ranges between 58,000- 60,000*u*S. This can explain the highest percent of false positive total coliform and *E. coli* reported by this study.

Statistical results:

ANOVA test detected that the presence of highly significance difference within the three methods tested for enumeration of both total and fecal coliform as presented in table (4). The multiple comparisons (LSD) as presented in table (5), recorded highly significance difference at 0.05 level between Colilert method and traditional MTF method in enumeration of total coliform, while no significance difference was recorded between Colilert and traditional MF method in enumeration of total coliform. This indicated that Colilert method was equivalent to the traditional MF method in enumeration of total coliform.

On the other hand, Colilert method was significantly different with the two traditional methods (MTF & MF) in enumeration of fecal coliform as represented in table (5).

Palmer *et al*.,[17] stated that there was

no difference between the Colilert and MTF for the detection of *E. coli* while for the detection of total coliform, the Colilert gave higher numbers than did the MTF method in their study on coastal water in southern California. They concluded that Colilert method is equivalent to the traditional MTF method for the detection of *E. coli* ,which uncomply with the present study, but not for the detection of total coliform which comply with the present study.

Covert[10] *et al*., stated a statistically significant difference in the number of positive tubes, with the MTF test resulting in more positive tubes.

Noble *et al*.,[15] stated a statistically significant difference between MTF and Colilert in detecting total coliform.

Compared with MTF, Colilert method showed values of sensitivity, specificity, and precision of, respectively, 25%, 90%, and 60% for detecting more positive fecal coliform. On the other hand, compared with MF, Colilert method showed values of sensitivity, specificity, and precision of, respectively, 16.7%, 75%, and 28% for detecting more positive fecal coliform.

These values indicated that the Colilert method was more closed to the MF than MTF. Colilert 18 is not recommended for the enumeration of total coliforms from marine water. Published studies suggest that there are substantial false positives, yielding higher total coliform counts from marine water.[17,20]

CONCLUSSION AND RECOMMENDATIONS

The study reported that Colilert method was more sensitive in detecting fecal coliform than the traditional methods (MTF and MF). No significance difference was detected between it and MF in enumeration of total coliform. The study recommended the following:

- The Colilert method was not acceptable in high salinity water due to the substantial of high rate of false positive and false negative results.
- The environmental laws must specify

the method used in enumeration of total and fecal coliform in marine water.

- If Colilert method is used, the limits must be higher than the standard traditional methods (MTF and MF).
- High dilution, (1/100), of samples must becarried out, if colilert method is used.
- The study recommended the use of MF method for the enumeration of coliform in such marine water and for marine water in general.

Table (1): The descriptive analysis of the results obtained by the different methods

		Geometric mean	Std. Error	Minimum	Maximum
Total coliform	**MTF**	1.8212	0.28996	0	5.04
	Colilert	3.2923	0.1327	2.3	5.4
	MF	2.8661	0.192	0	4.15
	Total	2.6599	0.13817	0	5.4
fecal coliform	**MTF**	0.2502	0.11876	0	2.48
	Colilert	0.7846	0.18369	0	2.81
	MF	0.1875	0.08616	0	1.93
	Total	0.4074	0.08226	0	2.81

Table (2): Concordance between Colilert and MTF and MF in detecting and undetecting total coliform

	MTF		MF	
	+Ve	-Ve	+Ve	-Ve
Colilert +Ve	87.5%	12.5%	90.4%	9.4%
Colilert –Ve	0%	0%	0%	0%

Table (3): Concordance between Colilert and MTF and MF in detecting and undetecting fecal coliform

	MTF		MF	
	+Ve	-Ve	+Ve	-Ve
Colilert +Ve	9.4%	25%	6.3%	31.3%
Colilert –Ve	6.3%	59.3%	15.6%	46.8%

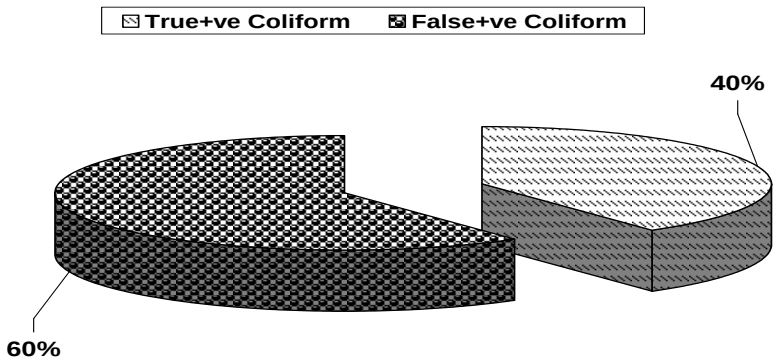
Table (4): Analysis of variance (ANOVA test)

		df	F	Sig.
Log total coliform count	**Between Groups**	2	12.406	0.000
	Within Groups	93		
	Total	95		
Log fecal coliform count	**Between Groups**	2	5.844	0.004
	Within Groups	93		
	Total	95		

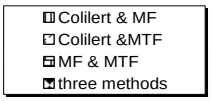
Table (5): the multiple comparison between the different methods (LSD)

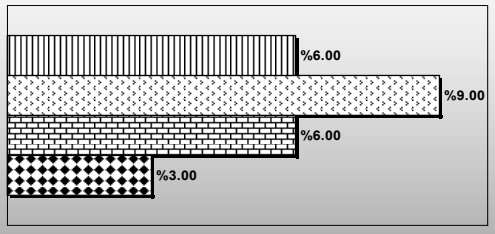
Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Log total coliform count	MTF	Colilert	-1.47114(*)	0.30392	0	-2.0747	-0.8676
		filtration	-1.04487(*)	0.30392	0.001	-1.6484	-0.4413
	Colilert	confirmed	1.47114(*)	0.30392	0	0.8676	2.0747
		filtration	0.42627	0.30392	0.164	-0.1773	1.0298
	MF	confirmed	1.04487(*)	0.30392	0.001	0.4413	1.6484
		Colilert	-0.42627	0.30392	0.164	-1.0298	0.1773
Log fecal coliform count	MTF	Colilert	-.53437(*)	0.19195	0.007	-0.9155	-0.1532
		filtration	0.06273	0.19195	0.745	-0.3184	0.4439
	Colilert	confirmed	.53437(*)	0.19195	0.007	0.1532	0.9155
		filtration	.59711(*)	0.19195	0.002	0.2159	0.9783
	MF	confirmed	-0.06273	0.19195	0.745	-0.4439	0.3184
		Colilert	-.59711(*)	0.19195	0.002	-0.9783	-0.2159

* The mean difference is significant at the .05 level.

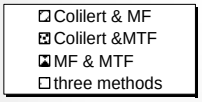


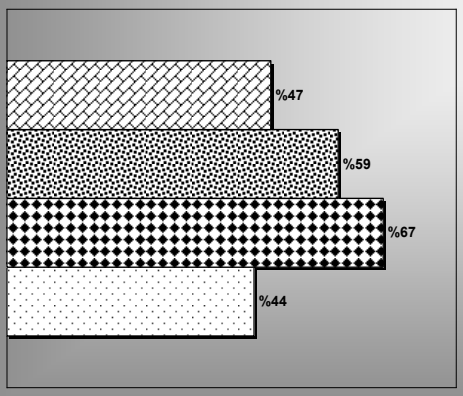
Figute (1): Results of conformatory tests carried out on positive total coliform detected by colilert method



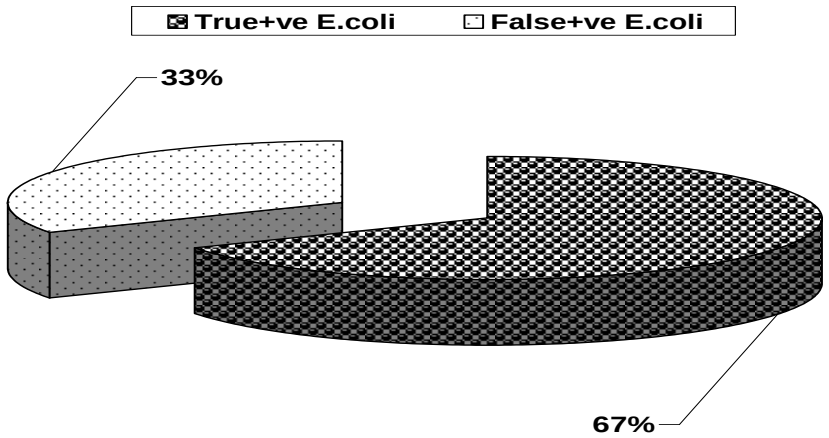


Figure(2): Concordance between the different methods of Detected Fecal Coliform





Figure(3): Concordance between the different methods of undetected Fecal Coliform



Figure(4):Results of conformatory tests carried out on positive *E.coli* detected by colilert method

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