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Evaluation of Commercial Colilert18-Quantitray® Method by ISO Techniques for Enumeration and Quantification of Total Coliforms and *Escherichia coli* in Drinking-Water of Buraidah, Saudi Arabia

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Abstract: In the present investigation, the Colilert18-QuantiTray® test is compared with ISO 9308-2:1999 Multiple-Tube Fermentation (MTF) and 9308-1:2000 Membrane Filtration (MF) methods for the detection of coliforms and *E. coli* in Buraidah drinking water. Regarding sensitivity and specificity of test methods using MTF as reference, the MF method showed a weak sensitivity, while the Colilert18® test showed the highest one. On the other hand, MF method exhibited a higher specificity compared with Colilert18® test. A moderately strong relationship among test methods of coliforms log transformed counts obtained with MF (cfu/100 mL) and Coliert18®/Quanti-Tray (MPN/100 mL) tests compared to MPN-MTF method was demonstrated. Regression analysis revealed the presence of strong linear correlations ($p<0.01$) between the three test methods and standard plate count of detecting the concentration of serially diluted *E. coli* LMG 2092 with high regression coefficients with MF and Colilert18® and lower value for MTF. The Colilert18® had *E. coli* detection down to 5 MPN/100 mL (≅ 0.05/1 mL). Results showed that Coliert18®/Quanti-Tray method is comparable with ISO methods further, the former method has more advantages such as higher sensitivity, maximum detection limit ability and time and labor saving.

Key words: Drinking water, coliforms, microbial indicators, *E. coli*, reference standard methods, Clilert18®-QunatiTray, mutable-tube fermentation, membrane filtration

INTRODUCTION

Water consumption increases in hot climates and in Saudi Arabia people consume large amounts of water for most part of the year. Diarrhoeal diseases, largely the consequence of faecal contamination of drinking-water supply are prevailing in the Kingdom. Diarrhoeal diseases are variously estimated to be responsible for 80% of morbidity/mortality, or more, in developing countries (World Health Organization, 2004). Therefore, public and environmental health protection requires that drinking water should be frequently monitored for the presence of pathogens. Understanding the origin of faecal pollution is paramount in determining associated health hazards as well as necessary corrective actions. Moreover, monitoring water quality is an indispensable GMP procedure inside dairy and food factories. For example, the water quality regulations of the Milk House in which milk is cooled or stored; or that contains milking equipments declared that water should be routinely tested. Water must be delivered contain less than 1 cfu/100 mL *Escherichia coli* bacteria and less than 10 cfu/100 mL total coliform bacteria.

Indicator microorganisms are frequently used to predict the presence of potential risks associated with pathogens. Indicator features of concern include nonpathogenic organisms that can be easily and rapidly detected and also those that have the ability to survive in the same environment of the pathogens of concern and thus can be strongly related with the presence of pathogenic organisms (Scott *et al*., 2002). Faecal coliforms exhibit typical characteristics of indicators and have been extensively used for several years as indicators for evaluating the sanitary conditions of water. Examination of faecal indicators, particularly for coliforms in drinking-water provides a very sensitive method for quality of water and in routine assessment of water (World Health Organization, 2004; Swistock and Sharpe, 2005). Von

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Fritsch, a German scientist initiated 125 years ago the characterization of certain bacteria to reflect human faecal contamination of water and from that time various typical methods have been developed to enumerate coliforms and *Escherichia coli* as indicators of water safety (Edberg *et al*., 1994).

These typical reference methods are usually performed in two formats, first is the Most Probable Number (MPN), second is Multiple-Tube Fermentation (MTF) based on lactose fermentation to produce acid and gas which was described in ISO 9308-2 (International Organization for Standardization, 1999). Other tests also depend on lactose fermentation and are collectively known as Membrane Filtration (MF) and were described in ISO 9308-1 (International Organization for Standardization, 2000). Both methods are prescribed in Saudi standards (Saudi Association of Standers Organization, 2000). Presumptive results are usually recorded after 24 to 48 h of incubation. Further, *E. coli* detection requires enumeration at elevated temperature (44.5°C) and testing for indole production. Consequently, quality control laboratories prefer to replace those time consuming and ineffective costly methods by easily performed, rapid and labor-saving with high sensitivity and specificity methods. Laboratories may use alternative tests in place of traditional ones to test the microbial quality of water and food, but they should demonstrate that results obtained are at least as reliable as those produced by using reference methods.

A new detection approach has been recently developed which depends on enzymatic reaction. Coliforms and *E. coli* possess different enzymes. This fact makes it possible to detect and differentiate between them. All the coliforms were found to be able to produce the enzyme β-D-galactosidase which degrades *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG), producing yellow-colored product o-nitrophenyl. Meanwhile, *E. coli* has a unique enzyme, β-D-glucuronidase that catalyzes the hydrolysis of chromogenic methylumbelliferyl-β-glucopyranoside (MUG) derivatives into the fluorescent product 4-methylumbelliferone (Novel and Novel, 1976; Rompré *et al*., 2002). The IDEXX Company developed the most widely used test among several commercial products known as Colilert® (IDEXX Labs, Westbrook, Maine, USA) which is based on defined substrate technology. Colilert® and Colilert18® simultaneously detect coliforms and *E. coli* in water within 24 and 18 h, respectively. More recently, the Colilert18®/Quanti-Tray system (IDEXX) was innovated, which is an extended MPN version of the Colilert®.

In Saudi Arabia like in other countries, governmental health monitoring and independent accredited laboratories may use commercial methods, for economic reasons, in place of ISO methods to analyze water quality. The interchangeability of these tests is highly important. Many agencies began adopting water quality standards based on *E. coli*. These new criteria must provide equivalent protection to that formerly provided by the ISO methods. Numerous comparative studies have shown that the obtained results by some commercially enzyme-based defined-substrate methods were comparable to those of the of the ISO MF method (Edberg *et al*., 1988; Clark and el Shaarawi, 1993; Eckner, 1998; Schets *et al*., 2002). However, the Coliert18®/Quanti-Tray system still need to be evaluated qualitatively and quantitatively in parallel with ISO 9308-2 (MTF) and ISO 9308-1 (MF) methods, the main reference methods used for the assessment of microbial quality of drinking water.

Buriadah is the capital of Qassim region, located in the center of Saudi Arabia, (28° 24N, 45° 41E) and considered to be the biggest agricultural area in the kingdom. The city water of Buriadah is amounted to be 85000 m^3 day^{-1} and produced from 23 wells with a pipelines network goes to 1876 km. The leakage of city water around the kingdom is estimated to be over 1.5 million m^3 (Public directorate of Qassim water), which reflects a high health hazard with potential cross-contamination. In addition, the sanitary conditions of household water weather in storage tanks or through the internal pipelines are still questionable especially, in desert climate with hot summer and cold winters.

One aim of the present study was to assess the microbial drinking water quality in houses and in public places (i.e., schools, public cold tanks and mosques) in Buraidah community, Saudi Arabia. Another aim was to evaluate the sensitivity and specificity of Colilert/Quanti-Tray® test in parallel with traditional ISO MTF and MF reference test methods

MATERIALS AND METHODS

Samples: Drinking water samples were collected according to standard procedure (Andrew *et al*., 2005), cooled and transported to the food microbiology laboratory, they were stored at 2-8°C and analyzed within 24 h. This survey took place in the period of 2006-2007. Samples were taken from different locations and sources in Buraidah. A total of 80 samples were analyzed throughout the study.

Microbiological analysis: Enumeration of total coliforms and *E. coli* was done using Colilert18® and Membrane-Filtration (MF) and Multiple-Tube Fermentation (MTF) as reference test methods. For MTF test the three successive

enumerating steps using Lauryl sulphate tryptose broth (LST; Oxoid, Basingstke, Hampshire, England), Brilliant green lactose bile (BGLB, Oxoid) and EC broth (Oxoid) was followed according to ISO 9308-2 (ISO, 1999). The MF test was done in consistency with ISO 9308-1 (ISO, 2000) on Tergitol 7 (LTTC; Merck, Darmstadt, Germany) using 0.45 μm pore size membrane filter (Schleicher and Schuell-Wattman Group Dassel, Germany). Colilert18® test was performed in accordance with the manufacture' instructions (IDEXX Labs, Westbrook, Maine, USA). In case of coliforms and *E. coli* counting, Quanti-Tray, which has a 96-well MPN format, was used. The developed yellow wells were further exposed to UV light (365 nm; C-70G Chromatovue, Upland, USA) to detect *E. coli* which give fluoresce if present.

Additional confirmatory tests: To assess the true positive rate and positive outcome results form each test were subjected to further confirmatory tests. Yellow-gassy BGLB tubes and EC tubes with gas in MTF test, yellow lactose-positive colonies from MF test and fluoresced cup or well in Colilert® Quanti-Tray test were initially inoculated on Tryptone Soya agar (TSA, Oxoid) and incubated at 37°C for 24 h. Colonies from the TSA plate were used for oxidase tests and was also inoculated into Tryptophane Broth (TB, Oxoid), then incubated for 24 h at 44.5°C and examined for the production of indole by adding 0.2-0.3 mL Kovacs' reagent. Lactose positive colonies being oxidase-negative were considered as total coliforms; lactose-positive colonies being oxidase negative, indole positive and produced gas at 44.5°C EC broth were considered *E. coli*. At all steps *E. coli* LMG 2092 and *Enterobacter aerogenes* LMG 2094 were used as reference strains.

Statistical evaluation: Sensitivity (true positive rate) was calculated by dividing the number of samples positive by the test evaluated and positive by the reference method by the number of samples positive by the reference method. Specificity (true negative rate) was calculated by dividing the number of samples negative by the test evaluated and negative by the reference method by the number of samples negative by the reference method.

A non-parametric Spearman's rank correlation coefficients with 2-tailed p-value were calculated for bivariate cross-correlations within the detected coliforms and *E. coli* with each test. To compare the *E. coli* counts obtained by using different methods, counts were compared pairwise after the logarithmic transformation of counts. Regression analysis and derived fitted models were performed to detect the accuracy of serially diluted *E. coli* LMG 2092 counted by each tests and compared with standard plate count with 95% CI. All statistical analysis was performed using SPSS 10.

RESULTS

General drinking water quality: Results of the detection (Presence/absence) of total coliforms and *E. coli* of Buraidah drinking water samples (n = 80) using a rapid Colilert18® test compared with MTF and MF as ISO reference test methods is shown in Fig. 1 and 2. Obtained results showed that the highest recovery of total coliforms was obtained with Colilert18® test (38.7%) followed by MF (31.3%) while MTF test had the lowest one (20.0%). With regard to the detection of *E. coli*, the Colilert18® test showed the same trend as was noticed for total coliforms with a maximum recovery of 13.7% however, there was no significant ($p>0.05$) different between MF (6.2%) and MTF (7.5%), ISO methods.

Through interpretation of the negative/positive results (absence/presence) of the target organisms as numerical (0) and (1) values, respectively, the correlation between tested methods could be calculated. Spearman rank correlation analysis using 2-tailed significance test revealed a powerful correlation between the three detection methods ($p<0.01$). Correlation coefficients of 0.56 and 0.80 between Colilert-MTF and Colilert-MF respectively, were obtained for coliforms. Similar correlation coefficients were observed for *E. coli* (0.52, Colilert18®-MTF and 0.71, Colilert18®-MF).

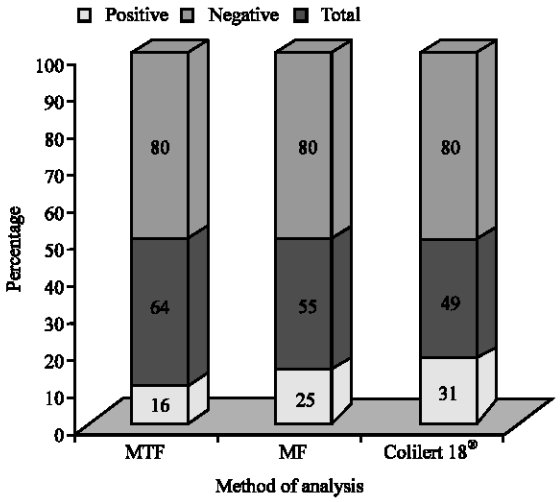

Fig. 1: Presence of total coliforms of Buraidah drinking water (n = 80) evaluated by Multiple Tube Fermentation (MTF), Membrane Filtration (MF) and Colilert 18® methods

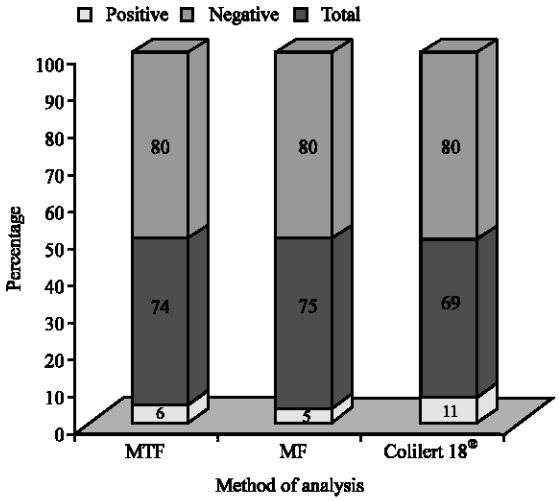

Fig. 2: Presence of *E. coli* of Buraidah drinking water (n = 80) evaluated by Multiple Tube Fermentation (MTF), Membrane Filtration (MF) and Colilert 18® methods

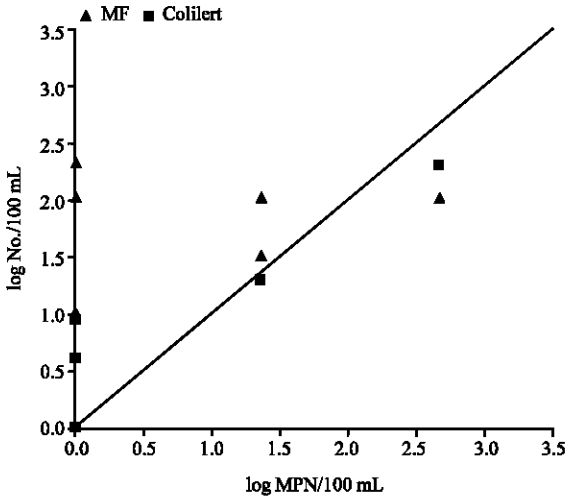

Fig. 3: Scatterplot analysis of log numbers of total coliforms in water samples enumerated by membrane filtration (MF, ISO 9308-1) and Colilert18® methods using multiple-tube fermentation test (MTF, ISO 9308-2) as reference method

Table 1: Levels of sensitivity and specificity of membrane filtration (MF, ISO 9308-1) and Colilert18® methods for enumeration of *E. coli* in drinking water samples using multiple-tube fermentation test (MTB, ISO 9308-2) as reference method

		Results by MFT				
Method	Results	No. of Ve-	No. of Ve+	No. of Total	Sensitivity (%)	Specificity (%)
MF,	Ve-	73	1	74		
Tergitol 7	Ve+	1	5	6		
(LTTC)	Total	74	6	80	83.3	98.6
Colilert18®	Ve-	69	0	69		
	Ve+	5	6	11		
	Total	74	6	80	100.0	86.3

Ve-: Negative, Ve+: Positive

Sensitivity and specificity for enumeration of *E. coli*: The sensitivity and specificity of the detection of *E. coli* in drinking water (n = 80) were challenged by ISO 9308-1 (MF) and Colilert18® tests in comparing with ISO 9308-2 (MTF-MPN) method (Table 1). As well known, these tests employed different amount of sample on the analysis. The MF method had weak sensitivity (83.3%) compared to Colilert® test (100%). Differently, MF method exhibited high specificity (98.6%) followed by Colilert18® test (86.3%).

Comparing count results in drinking water samples: A block of 11 collected samples were used to compare quantitatively the count of coliforms and *E. coli* using test methods. Results are displayed in simple in scatter chart with log transformed MPN-MTF counts on the X-axis as reference method, log transformed counts obtained with MF (cfu/100 mL) and Coliert18®/ Quanti-Tray (MPN/100 mL) tests on the Y-axis and a line of equality drawn in the graph. Figure 3 shows that MTF, MF and Colilert18® perform almost similar, except that one of colilert counts was above the liner of equality.

Statistical analysis showed correlation coefficients equal 0.78 and 0.73 between MTF-Colilert18® and MF-Colilert18® counts, respectively, indicating a moderately strong relationship among test methods. From the present coliforms counts, plots of fitted models had been derived out in order to explain the relation among the test methods and to predict the unknown counts by any test using detectable count evaluated by another one as shown in Fig. 4a and b. It is not possible to compare between *E. coli* counts using various test methods since the organism was detected only in three samples. The minimum count of *E. coli* detected was log 0.95/100 mL while the maximum is log 3.4 with a median of log 1.17.

Count accuracy levels and detectable limits: To assess the accuracy of counting and the minimum detection limits of test methods, a culture of *E. coli* LMG 2092 was serially diluted in filter-sterilized drinking water then enumerated with test methods in compared with Standard Plate Count (SPC). Scatterplot analysis was performed with log transformed SPC counts (cfu/100 mL) on the X-axis as reference method, log transformed counts obtained with MTF, MF and Coliert18®/Quanti-Tray tests on the Y-axis and a line of equality drawn in the graph (Fig. 5). The test methods showed a high level of consistency with standard plate count which was done on non-selective

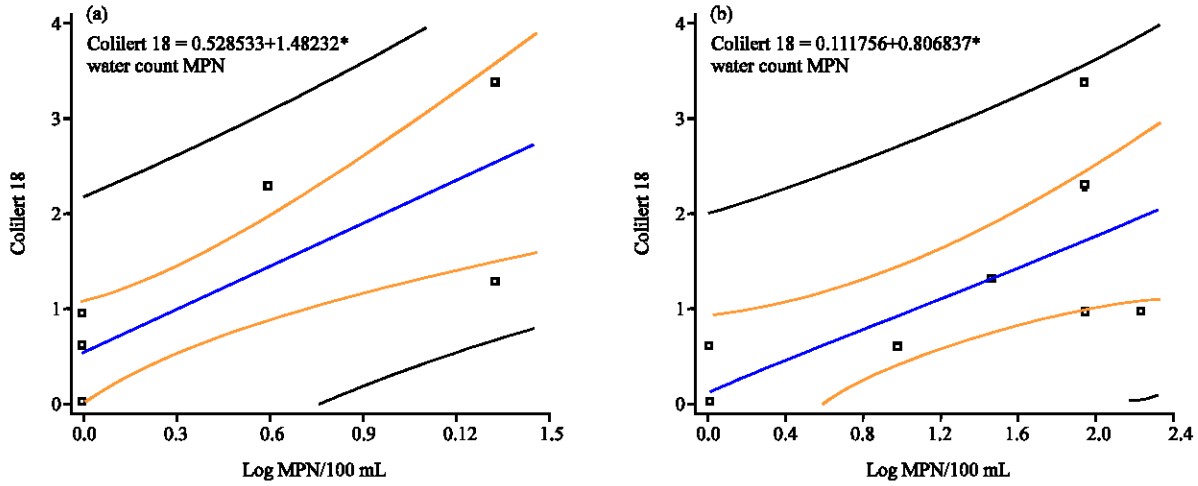

Fig. 4: Derived fitted Plot models of the relation between (a) Colilert18® and MTF (r^2 = 0.61%) and (b) Colilert18® and MF (r^2 = 0.53%) of total coliforms counts

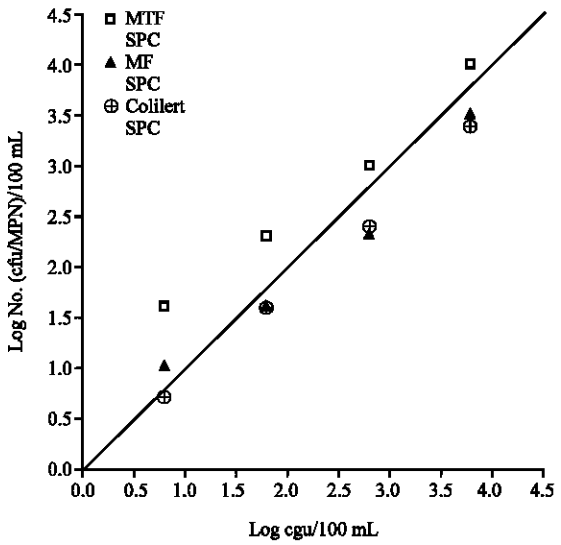

Fig. 5: Scatterplot analysis of log numbers of *E. coli* LMG 2092 serially diluted in filter-sterilized water enumerated by multiple-tube fermentation test (MTF, ISO 9308-2), membrane filtration (MF, ISO 9308-1) and Colilert18® methods compared to Standard Plate Count (SPC)

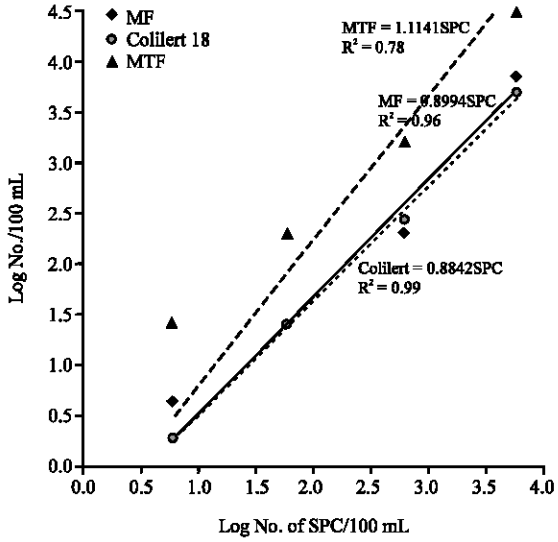

Fig. 6: Linear regression analysis of bacterial counts of *E. coli* LMG 2092 determined by multiple-tube fermentation test (MTF, ISO 9308-2), membrane filtration (MF, ISO 9308-1) and Colilert18® methods compared to Standard Plate Count (SPC)

medium (Tryptone Soya yeast extract agar), since all count points by the test methods lay around the line of equality (represents SPC counts).

The Colilert18® showed a minimum detection limit of *E. coli* of 5 MPN/100 mL (≅ 0.05/1 mL) which was comparable with MTF test where MF test limited by the amount of test sample (1 mL).

Regression analysis (Fig. 6) indicated presence of strong linear correlations between test methods and standard plate count ($p<0.01$) with high regression coefficients with MF and Colilert18® and lower value for MTF.

DISCUSSION

The present results for detection of coliforms and *E. coli* with ISO methods (MTF and MF) were comparable with the Colilert18® method. The nonparametric spearman correlation between Colilert18® method and reference

methods (MTF and MF) was significant for detection of total coliforms and *E. coli* in the drinking water samples. Findings in this study are in agreement with Fricker *et al.* (1997) and Eckner (1998), who reported strong correlation coefficients among test methods in drinking and surface waters. Meanwhile, the comparative present study was the only one compiled between the most known official used methods and Colilert18®.

The reagents of Colilert18® test attained sensitivity and specificity similar to those of MTF and MF tests. However, the Colilert18® method detected higher positive coliforms and *E. coli* samples. Similar results were reported by Hörman and Hänninen (2006).They reported that Colilert18® test method had great sensitivity but with low specificity for detecting *E. coli* in surface water.

The designed study showed the high ability of Colilert18® to detect as minimum as possible of *E. coli* of goes down to 5 MPN/100 mL (≅ 0.05/1 mL). High recovery by Colilert18® is most probably due to the ability to induce healing of stressed cells (McFeters *et al*., 1995). Beside the limited sample volume (1 mL) which failed to attain the sensitivity of MF procedure, it is known that MF reduces the recovery of detected organisms (Brenner and Rankin, 1990). In the present study, there was a high agreement ($p<0.01$) between the obtained counts of serially diluted unstressed *E. coli* LMG 2092 by the three test methods compared with Standard Plate Count (SPC). However, retrogression coefficient between MTF and SPC might be attributed to the inhibitory effects of selective reagents included in the media used in the MTF procedures.

The differences between the ISO methods and Colilert18® test could be explained by the different substrates employed, resulting in the detection of two different groups of coliform bacteria. Coliforms need two enzymes to produce yellow color in lactose-containing media. First one is β-galactoside-premease, for active lactose transport into the bacterial cell, while the second, β-galactosidase, to hydrolyze lactose resulting in the production of acid and gas. The gene codes of these enzymes are *LacY* and *LacZ*. Coliforms that lack of *LacY* gene do not produce yellow color in lactose containing media however, in the presence of *LacZ* gene the Colilert substrate of *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) can be used and produce yellow color Fricker *et al.* (1997) reported that a larger population of coliform bacteria (10% of isolates) contains only the *LacZ* gene and did not ferment lactose due to lake of β-galactoside-premease. Consequently, using the same defined substrate (lactose) and confirmation protocol in MTF and MF methods could give rise to identical results. As Colilert18® test and ISO methods (MTF and MF) enumerated different groups of coliforms, detected counts by these methods could be fairly compared when results are confirmed and identical criteria used to define group known as total coliforms are typical. Hence, from the present results it is suggested that larger number of lactose fermenting total coliforms will be detected with Colilert18® test than with ISO methods (MTF and MF).

CONCLUSION

It can be concluded from, this study that the use of Colilert18-QuantiTray® is suitable for evaluation of drinking water quality due its sensitivity and maximum detection limit ability and because it features time and labor saving and can be easily performed. However, confirmatory tests should be performed to overcome the handicap of specificity behavior in Colilert18-QuantiTray® test. Using these methods indicated that Buraidah drinking water suffers from critical hygienic problems which demands further attention to control the post-contamination at houses inside storage tanks.

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