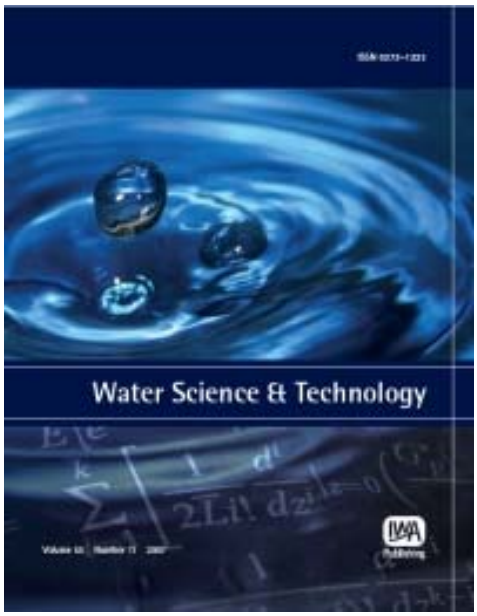

Equivalency testing of TTC Tergitol 7 agar (ISO 9308-1:2000) with five culture media for the detection of *E. coli* in water samples in Greece

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ABSTRACT

In this study ten laboratories in Greece compared the performance of reference method TTC Tergitol 7 Agar (with the additional test of β-glucuronidase production) with five alternative methods, to detect *E. coli* in water, in line with European Water Directive recommendations. The samples were prepared by spiking drinking water with sewage effluent following a standard protocol. Chlorinated and non-chlorinated samples were used. The statistical analysis was based on the mean relative difference of confirmed counts and was performed in line with ISO 17994. The results showed that in total, three of the alternative methods (Chromocult Coliform agar, Membrane Lauryl Sulfate agar and Trypton Bilex-glucuronidase medium) were not different from TTC Tergitol 7 agar (TTC Tergitol 7 agar vs Chromocult Coliform agar, 294 samples, mean RD% 5.55; vs MLSA, 302 samples, mean RD% 1; vs TBX, 297 samples, mean RD% −2.78). The other two alternative methods (Membrane Faecal coliform medium and Colilert 18/ Quantitray) gave significantly higher counts than TTC Tergitol 7 agar (TTC Tergitol 7 agar vs MFc, 303 samples, mean RD% 8.81; vs Colilert-18/Quantitray, 76 samples, mean RD% 18.91). In other words, the alternative methods generated performance that was as reliable as, or even better than, the reference method. This study will help laboratories in Greece overcome culture and counting problems deriving from the EU reference method for *E. coli* counts in water samples.

Key words | equivalency testing, *Escherichia coli*, Greece, microbiological methods, water

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INTRODUCTION

The detection and enumeration of *E. coli* in water samples have traditionally been based either on the multiple tube fermentation (MTF) method using the most probable number (MPN) estimation of the bacterial count or on membrane filtration (MF) methods (Rompre *et al.* 2002). Using MF methods, "presumptive" colonies are plated onto nutrient agar and examined for the production of cytochrome oxidase, their ability to ferment lactose at 37°C and 44°C and their ability to produce indole from tryptophan using tryptone water and Kovacs reagent. However, no single method relying on these tests is able to recover all strains of a particular organism or group of organisms. The occurrence of strains of *E. coli* which are negative for one or more of these traits is higher than the occurrence of strains which are negative for β-D-glucuronidase (Niemelä *et al.* 2003). In recent decades new chromogenic or fluorogenic defined substrate methods based on β-D-glucuronidase (for the detection of *E. coli*) have been introduced (Ashbolt *et al.* 2001; Hörman & Hänninen 2006). Because of differences in test principles the outcome of different methods may vary in respect of the numbers of organisms detected, and the tests may also detect metabolically differing types of organisms (Ashbolt *et al.* 2001; Rompre *et al.* 2002).

The European Drinking Water Directive (98/83/EC, 1998) defines reference methods for the enumeration of microbiological parameters in drinking water. The method defined for enumeration of total coliforms and *E. coli* is MF on Lactose TTC agar with Tergitol 7 (Chapman 1951) as described in ISO 9308-1:2000. In 2007, a technical corrigendum to this standard was published recommending the additional use of the β-D-glucuronidase test. Fricker *et al.* (2008) suggest that use of the test for the detection of β-D-glucuronidase as a marker for *E. coli* gives more accurate results than use of tests for indole production at 44°C. Nevertheless, the Directive specifies that "Member States which have recourse to alternative methods shall provide the Commission with all relevant information concerning such methods and their equivalence". A method is normally considered "equivalent" if the (confirmed) recovery of target organisms is not significantly different from the reference method. For the purposes of this study, and in accordance with ISO methods comparison protocols (ISO 17994), the test methods were considered to be equivalent to the reference method if the mean difference (MD) in recovery of target organisms was not significantly different from zero. Several equivalence studies have been published based on the comparison protocol described in ISO 17994 (2004) (Niemelä *et al.* 2003; Bernasconi *et al.* 2006; Bonadonna *et al.* 2006; Chao 2006).

In Greece there are no nationally prescribed methods for the microbiological analysis of water. A survey carried out among laboratories participating in the proficiency testing scheme EQUASE – Greece (1996 – 2005) showed that most laboratories noted drawbacks in the use of the TTC Tergitol 7 agar method. Readability of results and time to get the response seem to be the main limitations of the method. The same survey demonstrated that for the enumeration of *E. coli* in water samples, 41.2% of the laboratories were using TTC Tergitol 7 agar, 29.4% Membrane Lauryl Sulfate agar, 11.7% Tryptone Bilex-glucuronidase medium, 11.7% Membrane Faecal coliform medium and 6% Chromocult Coliform agar. In recent years Colilert-18/Quantitray has been widely used by many laboratories worldwide (Fricker *et al.* 1997; Bonadonna *et al.* 2006). Many Greek laboratories are currently considering its use.

This study was undertaken to compare the performance of the commonly used culture media by Greek laboratories for the detection of *E. coli* in water samples, including Colilert-18/Quantitray, with the ISO 9308-1:2000 method specified by the European Directive (with the additional confirmation test for the detection of β-D-glucuronidase), with a view to introducing new methods to Greek labs and addressing problems in the reference method. This study provides comparison data for laboratories seeking to adopt, for routine use, alternatives to the reference method for the detection of *E. coli*.

MATERIALS AND METHODS

Participating laboratories

A total of ten laboratories located in various parts of Greece participated in the study: Athens, three laboratories, Northern Greece-Thessalonica, two laboratories, Northern

Greece-Thrace, one laboratory, Southern Greece-Crete, three laboratories, Eastern Greece-Rhodes, one laboratory. The selection of the laboratories was based on their good performance over 10 years participation in the proficiency testing scheme EQUASE (Extension of Quality Assurance in Water Microbiology to Cohesion Countries, http://www.watermicro.gr). All the laboratories are accredited according to ISO 17025 (2005) by the National Accreditation Body of Greece (ESYD).

Samples

Each laboratory chose the type of water sample to be used in this study based on their experience. The samples were prepared by spiking drinking water with sewage effluent following a standard protocol based on the procedures described in "The Microbiology of Drinking Water–Part 3" (Standing Committee of Analysts 2002). The day before the experiment sewage samples were collected and stored in the refrigerator to allow precipitation of dissolved solids. Chlorinated water samples were produced by inoculating chlorinated tap water with sewage effluent. Furthermore, extra quantities of chlorine were added to a final concentration of between 0.1 and 0.5 mg/L to produce chlorine-stressed organisms. A preliminary trial was carried out to determine the level of chlorine required in the samples. Chlorination was conducted so that total counts of presumptive target bacteria would be approximately in the range of 10–90 cfu/100 mL. Non-chlorinated drinking water samples were prepared by adding appropriate volumes of sewage effluent in non-chlorinated drinking water so that total counts of presumptive target bacteria would be approximately in the range of 10–90 cfu/100 mL.

Microbiological methods

The reference method for the comparisons uses TTC Tergitol 7 agar (ISO 9308-1, 2000) with the additional confirmation test for the detection of β-D-glucuronidase in accordance with ISO 9308-1:2000/Cor.1:2007. This additional test is recommended in order to prevent false positive results deriving from the presence of indole positive *Klebsiella oxytoca* strains in the samples.

The reference method with the additional confirmation test was compared with five methods applied widely by Greek laboratories (Table 1). The media were chosen for their ability to differentiate *E. coli* colonies, allow detection at low and high concentrations, inhibit non-target microorganisms and enhance injured

Table 1 | Evaluated test methods for detection of *Escherichia coli* in water samples. All media are selective, detecting typical colonies. All test methods are quantitative

Test method (reference)	Incubation	Interpretation, confirmation
TTC Tergitol 7 agar (ISO 9308-1)	36 ± 2°C/21 ± 3 h	yellow or yellow-green, oxidase-negative, produce acid from lactose, produce indole from tryptophan at 44.0 ± 0.5°C
		β-glucuronidase production Colilert medium 36 ± 2°C 18 h
Membrane Faecal coliforms agar (APHA 9222D 1998)	44.5 ± 0.2°C/24 ± 2 h	various shades of blue, oxidase-negative, produce acid from lactose, produce indole from tryptophan at 44.0 ± 0.5°C
Chromocult Coliform agar	36 ± 1°C/24 ± 2 h	dark-blue to violet colonies (Salmon-GAL and X-glucuronide reaction),
		positive indole formation after coating the typical colonies with KOVACS' indole reagent
Membrane Lauryl Sulfate agar (HPA,W2,2005)	44.5 ± 0.2°C/24 ± 2 h	yellow colonies, oxidase-negative, produce acid from lactose, produce indole from tryptophan at 44.0 ± 0.5°C
TBX (Tryptone Bile x-glucoronide) (ISO 16649-1)	44°C/4 ± 3 h	blue/green colonies, oxidase-negative, produce acid from lactose, produce indole from tryptophan at 44.0 ± 0.5°C
Colilert 18/Quantitray (DWI 2000)	36 ± 2°C/18 h	yellow colour with fluorescence, oxidase-negative, produce acid from lactose, produce indole from tryptophan at 44.0 ± 0.5°C
		β-glucuronidase production Colilert medium 36 ± 2°C 18 h

E. coli. These test media were also tested for their ability to differentiate non-target but closely related microorganisms:

- Membrane Faecal coliform medium (MFc)
- Chromocult Coliform agar (Merck, chromogenic medium)
- Membrane Lauryl Sulfate agar (MLSA)
- Tryptone Bilex-glucuronidase medium (TBX, chromogenic medium) and
- Colilert 18/Quantitray (IDEXX Colilert-18 with 51 wells quantitray, chromogenic/fluorogenic medium).

The MF technique was applied for the detection and enumeration of *E. coli* using the above media (except for Colilert 18/Quantitray, which is based on the MPN technique). For MLSA, MFc, TBX, and Chromocult Coliform agar, 100 mL and appropriate decimal dilutions were filtered through 0.45 μm membranes. The selection of typical colonies for identification was a critical factor. Colonies with a different morphology from that described by each method were ignored, as this is the practice in routine analyses. For Colilert 18/Quantitray, the manufacturer's instructions were followed. Subculturing of presumptive colonies was performed on MacConkey agar, and typical lactose positive colonies were confirmed.

Number of samples

The number of participating laboratories and the total number of samples were calculated according to the requirements of ISO 17994 for the establishment of equivalence between quantitative microbiological methods.

Each laboratory examined more than 30 samples using three of the four media (MLSA, MFc, TBX, Chromocult Coliform agar) in parallel with the reference method. In this trial, verification was carried out by just one laboratory, which tested 76 samples using Colilert 18/Quantitray in parallel with the reference method, as the Colilert 18/Quantitray method has been tested in other collaborative trials (Niemelä *et al.* 2003; Bonadonna *et al.* 2007) and officially accepted by many countries (testing requirements as per ISO 17994, paragraph 5.3.8).

Statistical analysis

The statistical analysis proposed by ISO 17994 is based on the average relative difference (RD) of confirmed counts (on natural logarithmic scale) and uses parametric statistical methods. However, lack of normality is common for microbiological data even after the natural logarithmic transformation. In addition to the ISO 17994 approaches, non-parametric methods or a combination of parametric and non-parametric tests are frequently used for analysis of microbiological data (DWI 2000; Schets *et al.* 2002; Pitkänen *et al.* 2007).

In the present study, the statistical analysis was performed according to ISO 17994 using parametric methods unless deviations from normality were observed.

The Shapiro-Wilk Normality test (Shapiro & Wilk 1965) was performed for the RD% of every comparison between the reference method and the alternative method. Since this normality test was not significant at the 0.05 significance level, the statistical analysis proposed by the ISO 17994 was used for the evaluation of the comparison between an alternative method and the reference method in each laboratory.

The expanded uncertainty (U) was obtained by multiplying the standard error of the mean RD by the coverage factor $k = 2$. To evaluate the result of the comparison, the "confidence interval" of the expanded uncertainty around the mean RD was calculated.

The alternative method was considered acceptable when its average performance was either quantitatively equivalent to or higher than the reference method. The alternative method was considered to give significantly higher counts than the reference method if the confidence interval of the expanded uncertainty around the mean RD lay entirely above zero. The methods were considered quantitatively equivalent ("not different") if the mean RD did not differ significantly from zero and the lower limit of the expanded uncertainty was not smaller than the lower value $-D = -10\%$ of the "maximum acceptable deviation". That means that the alternative method could not present more than 10% worse recovery of *E. coli* than the reference method. If the expanded uncertainty covers both zero mean RD and the -10% acceptable deviation, the comparison is considered to be inconclusive and more samples should be examined.

The independent samples *t*-test or the non-parametric Mann-Whitney test was used to evaluate differences in RD% between chlorinated and non-chlorinated samples.

To compare the RD% between the laboratories, one-way ANOVA with the Brown-Forsythe procedure (Brown & Forsythe 1974) (to take into account the variance heterogeneity) and pair-wise comparison by the Tamhane's T2 test were used Alternatively to the one-way ANOVA with the Brown-Forsythe procedure, the non-parametric test of Kruskal-Wallis with Monte-Carlo significance was used.

The Shapiro-Wilk Normality test was also performed for the RD% of the pooled data of all the laboratories in each comparison between the reference method and an alternative one. In the case that the overall data was not normally distributed, the evaluation was based on the 95% confidence interval for the median of the paired counts differences, which was estimated using the Wilcoxon signed rank test (Lehmann 1975), instead of the confidence interval of the mean RD.

Comparisons were only performed between the reference method and the alternative methods. No comparisons between two or more alternative methods were performed.

The majority of the statistical analysis was performed with the statistical package SPSS 13.0 for Windows. The Wilcoxon signed rank test was performed with the statistical package Minitab 14.

RESULTS & DISCUSSION

Overview

Five alternative methods were compared with the reference method. The total number of comparisons made (derived from 462 samples analysed by ten different laboratories) amounted to 1,272. Table 2 identifies which methods were compared in which laboratories. No confirmed counts with zero value were observed. For all comparisons, there was not found a statistically significant difference in the mean RD% between chlorinated and non-chlorinated samples. Therefore, the results concern all samples (chlorinated and non-chlorinated combined).

A preliminary investigation of the data is given by plotting the natural logarithmic transformed counts by each one of the alternative methods vs. the natural logarithmic transformed counts by the reference method (Figure 1). Figure 1 shows that most of the data points of one laboratory were below the line of equivalence in every comparison this laboratory participated in. Nevertheless, there was not found a systematic error, lack of training or other reason implying the exception of this laboratory from the statistical analysis. Furthermore, the interpretation of the whole dataset was

Table 2 | Number of comparisons derived from the tests of non-chlorinated (chlorinated) samples per participating laboratory, and per alternative method vs. reference method

	Alternative methods compared to TTC Tergitol 7 agar					
Laboratory	**MFc**	**Chromocult**	**MLSA**	**TBX**	**Colilert**	**Total**
1	34 (0)	32 (0)	33 (0)	30 (0)	76 (0)	205 (0)
2	28 (10)	28 (9)	28 (9)			84 (28)
3		39 (0)	39 (0)	39 (0)		117 (0)
4	29 (8)		29 (8)	28 (8)		86 (24)
5	38 (0)	38 (0)		38 (0)		114 (0)
6	27 (9)	55 (19)	28 (10)			110 (38)
7		38 (0)	38 (0)	37 (0)		113 (0)
8	38 (0)		38 (0)	38 (0)		114 (0)
9	25 (12)	24 (12)		25 (12)		74 (36)
10	36 (9)		33 (9)	33 (9)		102 (27)
Total	255 (48)	254 (40)	266 (36)	268 (29)	76 (0)	1119 (153)

MFc: Membrane Faecal coliform agar, MLSA: Membrane Lauryl Sulfate agar, TBX: Tryptone Bile x-glucuronide.

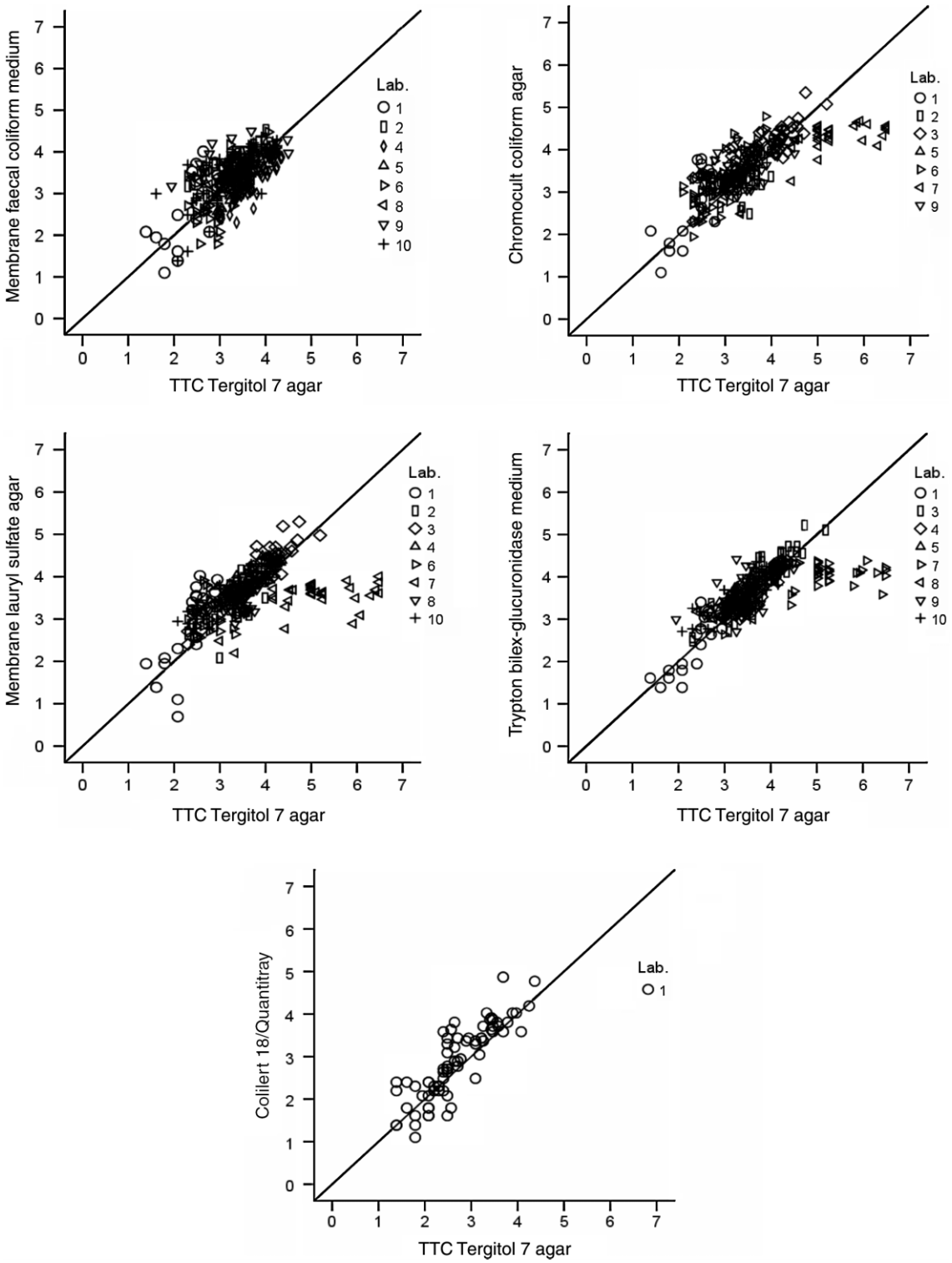

Figure 1 | Scatter plots of the confirmed counts by the test method vs. confirmed counts by TTC Tergitol 7 agar (on natural logarithmic scale).

not being affected by the participation of this laboratory. Therefore, all laboratories were included to the final data.

Eight laboratories performed 303 "MFc vs. TTC Tergitol 7 agar" comparisons in total. Overall, the confirmation rates were 79.5% and 44.9% for the MFc and the TTC Tergitol 7 agar methods respectively.

Seven laboratories performed 294 "Chromocult Coliform agar vs. TTC Tergitol 7 agar" comparisons in total. Overall, the confirmation rates were 94.3% and 45.0% for the Chromocult Coliform agar and the TTC Tergitol 7 agar respectively.

Eight laboratories performed 302 "MLSA vs. TTC Tergitol 7 agar" comparisons in total. Overall, the confirmation rates were 83.9% and 53.4% for the MLSA and the TTC Tergitol 7 agar method respectively.

Eight laboratories performed 297 "TBX vs. TTC Tergitol 7 agar" comparisons in total. Overall, the confirmation rates were 96.2% and 49.0% for the MLSA and the TTC Tergitol 7 agar method respectively.

One laboratory performed 76 "Colilert 18/ Quantitray vs TTC Tergitol 7 agar" comparisons. The confirmation rates were 64.9% and 25.0% for the Colilert 18/ Quantitray and the TTC Tergitol 7 agar method respectively. In this comparison the confirmation rate of Colilert 18/Quantitray seems rather low, as also does the rate of TTC Teritol 7. This may be explained by the fact that this comparison was performed by only one laboratory and only for non-chlorinated samples.

Overall, confirmation rates of all the alternative methods were higher than the rates observed for the reference method. This can be explained by the differences in the methods' principles (e.g. chromogenic/fluorogenic differentiation, incubation temperature, selectivity and specificity of the media).

MFc vs. TTC Tergitol 7 agar

Both the ANOVA and the non-parametric Kruskal-Wallis test indicated significant differences in the mean RD% between laboratories (p-value < 0.001). The Normality test for the overall RD% was not significant at the 0.05 significance level. The comparison of the methods (Table 3) indicated that the average recovery of *E. coli* was statistically significantly higher (mean RD% = 8.81) using the MFc method than the reference method TTC Tergitol 7 agar (one sample t-test for RD%, H_0: mean RD% ≤ 0, H_1: mean RD% > 0: p-value $= 0.001$).

MFc medium is recommended by APHA (1998) for the detection and enumeration of *E. coli* from water samples, and, according to our results, it may be used as an alternative method to TTC Tergitol 7 agar.

Chromocult Coliform agar vs. TTC Tergitol 7 agar

Both the ANOVA and the Kruskal-Wallis test indicated significant differences in the mean RD% between laboratories (p-value < 0.001).

The overall mean RD% was 5.55 and the two methods were "not different" (Table 3).

Since the Shapiro-Wilks test was significant (p-value < 0.05) for the overall RD%, the 95% confidence interval for the overall median of the paired count differences was estimated using the Wilcoxon signed rank test.

Table 3 | Summary of the analysis of RD% of each alternative method vs. TTC Tergitol 7 agar for confirmed counts of *E. coli*

Alternative method	N	Mean RD%	Std. Dev.	Std. Error	Expanded uncertainty interval U	P-value*	LO	HI	Evaluation
MFc	303	8.81	49.24	2.83	5.66	0.002	3.15	14.47	†
Chromocult Coliform agar	294	5.55	57.15	3.33	6.67	0.097	−1.12	12.22	Not different
MLSA	302	1	74.87	4.31	8.62	0.818	−7.63	9.61	Not different
TBX	297	−2.78	57.42	3.33	6.66	0.404	−9.44	3.88	Not different
Colilert 18/Quantitray	76	18.91	44.18	5.07	10.13	<0.001	8.78	29.05	†

*H_0: Mean RD% = 0 vs; H_1: Mean RD% ≠ 0.

†significant positive difference (the alternative method has a significantly higher recovery than the reference method); Not different, methods are not statistically "different".

N, number of comparisons; Std. Dev., Standard Deviation; U, expanded uncertainty; LO, lower limit of the expanded interval uncertainty; HI, upper limit of the expanded interval uncertainty; MFc: Membrane Faecal coliform agar; MLSA: Membrane Lauryl Sulfate agar; TBX: Tryptone Bile x-glucuronide.

The confidence interval was 1.0, 4.0. Since the confidence interval did not include zero, we can conclude that (at the 0.05 significance level) Chromocult Coliform agar presented a better recovery of *E. coli* than the reference method (Wilcoxon signed rank test for the paired count differences (Chromocult Coliform agar - TTC Tergitol 7 agar), H_0: median difference ≤ 0, H_1: median difference >0: p-value $= 0.004$).

Previous studies have reached analogous conclusions. Chromocult Coliform agar was found to be no different from TTC Tergitol 7 agar in a Dutch study (Schets *et al.* 2002). Hamilton *et al.* (2006) and Bonadonna *et al.* (2007) report that Chromocult Coliform agar is more sensitive than the ISO reference procedure.

Chromocult Coliform agar was used, because the application of defined substrate medium technology with particular selective growth conditions and the simultaneous detection of β-D-glucuronidase activity have become widespread tools for the detection of *E. coli* in water and wastewater, allowing emerging problems to be detected and corrected earlier. This method minimises time, labour and expense of repeat or serial analyses which can delay the detection of contaminated drinking water.

MLSA vs. TTC Tergitol 7 agar

Both the ANOVA and the Kruskal-Wallis test indicated significant differences in the mean RD% between laboratories (p-value < 0.001).

The overall mean RD% was 1 and the two methods were "not different" (Table 3).

Due to the significant Shapiro-Wilks test (p-value < 0.05) for the overall RD%, the 95% confidence interval for the overall median of the paired count differences was estimated using the Wilcoxon signed rank test. The confidence interval was 3.0–6.5. Since the confidence interval did not include zero, we can conclude that (at the 0.05 significance level) MLSA presented a better recovery of *E. coli* than the reference method (Wilcoxon signed rank test for the paired count differences (MLSA - TTC Tergitol 7 agar), H_0: median difference ≤ 0, H_1: median difference > 0: p-value < 0.001).

The results of the present study confirm the results of other inter-laboratory studies. The MLSA method has been proven to be more selective than TTC Tergitol 7 agar in the Netherlands (Schets *et al.* 2002). According to the Dutch study, MLSA can be used for analysis of water samples of various contamination levels.

TBX vs. TTC Tergitol 7 agar

Both the ANOVA and the Kruskal-Wallis test indicated significant differences in the mean RD% between laboratories (p-value < 0.001). The overall mean RD% was -2.78 and the two methods were "not different" (Table 3).

Due to the significant Shapiro-Wilks test (at the 0.05 significance level) for the overall RD%, the 95% confidence interval for the overall median of the paired count differences was estimated using the Wilcoxon signed rank test. The confidence interval was 0.0–3.5. Since the confidence interval did not include negative values, we can conclude that (at the 0.05 significance level) TBX presented equal or better recovery of *E. coli* than TTC Tergitol 7 agar (Wilcoxon signed rank test for the paired count differences (TBX - TTC Tergitol 7 agar), H_0: median difference ≤ 0, H_1: median difference > 0: p-value $= 0.019$).

Numerous comparisons have shown that TBX medium, like many chromogenic substrates, may be a suitable alternative to the classical techniques and to TTC Tergitol 7 agar (Rompre *et al.* 2002).

Colilert 18/Quantitray vs. TTC Tergitol 7 agar

The summary of the analysis of RD% of Colilert 18/Quantitray vs. TTC Tergitol 7 agar for confirmed values for *E. coli* is presented in Table 3. The results show that the mean RD% is statistically significantly greater than zero (one sample t-test for RD%, H_0: mean RD% ≤ 0, H_1: mean RD% > 0: p-value < 0.001), indicating that the Colilert 18/Quantitray method has a significantly higher recovery than the TTC Tergitol 7 agar.

Various studies have compared Colilert 18/Quantitray with the EU reference method (TTC Tergitol 7 agar), and agree with the outcome of our study. Bernosconi *et al.* (2006) concluded that the EU reference method failed to detect a high percentage of *E. coli* colonies, while Colilert 18/Quantitray provided results in a shorter time and enabled the simultaneous detection of *E. coli* with no

further confirmation steps. In a study conducted by Hörman & Hänninen (2006) Colilert 18/Quantitray gave significantly higher counts for *E. coli* than the TTC Tergitol 7 agar method. Niemelä *et al.* (2003) concluded that Colilert 18/Quantitray is a suitable alternative to the EU reference method for the detection of *E. coli* in water. Bonadonna *et al.* (2006; 2007) also report that Colilert 18/Quantitray detects a higher number of target microrganisms versus the European reference method.

Major limitations of the membrane filtration method using TTC Tergitol 7 agar are that it takes up to 2 days to obtain results, it is labour intensive and does not identify *E. coli* directly. The use of 4-methylumbelliferyl-beta-D-glucuronide (MUG) for the detection of *Escherichia coli* (Colilert 18/Quantitray) offers the benefit of reducing the workload by simply counting the number of fluorescent tubes, without additional confirmation testing.

CONCLUSIONS

The detection of *E. coli* in drinking water is crucial for water providers, health care professionals and regulators. Consequently, the choice of methodology for detecting these organisms is of paramount importance.

The European Drinking Water Directive specifies that Lactose TTC agar with Tergitol 7 should be used for the examination of drinking water for regulatory purposes unless member states supply specific data to demonstrate that an alternative method produces comparable results.

In this Greek inter-laboratory study, participants pointed out two important drawbacks of the reference method: the low readability of results and the length of time required to obtain definitive response to analyses, in comparison with the alternative methods used in this study. These findings apply when either chlorinated or non-chlorinated samples were used.

In conclusion, MFc medium, MLSA, TBX medium, and Chromocult Coliform agar are potential alternative methods for detection of *E. coli* from waters with variable microbial load, since all these methods generated performance that was as reliable as, or even better than, the reference method. This study indicates that, for non-chlorinated water samples, the Colilert 18/Quantitray system is a good alternative to the reference method.

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