# Evaluation trials for two media for the simultaneous detection and enumeration of *Escherichia coli* and coliform organisms 1998

Methods for the Examination of Waters and Associated Materials

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# About this series

## Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, groundwater, river and seawater, waste water and effluents as well as sewage sludges, sediments and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

## Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests reported for most parameters. These methods should be capable of establishing within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results encompassing at least ten degrees of freedom from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors), systematic error (bias), total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available. An indication of the status of the method is shown at the front of this publication on whether or not the method has undergone full performance testing.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

## Standing Committee of Analysts

The preparation of booklets in the series 'Methods for the Examination of Waters and Associated Materials' and their continuous revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are:

| | |
|---|---|
| 1.0 | General principles of sampling and accuracy of results |
| 2.0 | Microbiological methods |
| 3.0 | Empirical and physical methods |
| 4.0 | Metals and metalloids |
| 5.0 | General non-metallic substances |
| 6.0 | Organic impurities |
| 7.0 | Biological monitoring |
| 8.0 | Sewage treatment methods and biodegradability |
| 9.0 | Radiochemical methods |

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members associated with this method are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and the more important parameters and topics is available from HMSO (ISBN 0 11 752669 X).

Every effort is made to avoid errors appearing in the published text. If, however, any are found please notify the Secretary.

Dr D Westwood
*Secretary*

October 1997

# Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with The Health and Safety at Work etc Act 1974 and any regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1988 (SI 1988/1657). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety, and these should be consulted and be readily accessible to all analysts. Amongst such publications are those produced by the Royal Society of Chemistry, namely 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', 5th edition, 1992; by Member Societies of the Microbiological Consultative Committee, 'Guidelines for Microbiological Safety', 1986, Portland Press, Colchester; and by the Public Health Laboratory Service 'Safety Precautions, Notes for Guidance'. Another useful publication is produced by the Department of Health entitled 'Good Laboratory Practice'.



# Evaluation trials for two media for the simultaneous detection and enumeration of *Escherichia coli* and coliform organisms

## 1 Summary

The use of specific substrates for the detection of *Escherichia coli* (*E. coli*) and coliform organisms is becoming increasingly popular. Several formulations using agar and broth-based media for the recovery of these organisms from water have been published. The general principle depends upon the detection of ß-glucuronidase for the specific detection of *E. coli*, and the detection of ß-galactosidase for the specific detection of coliform organisms. This booklet presents the results of evaluations of two media. Firstly, an agar-based membrane filtration (MF) medium, namely membrane lactose glucuronide agar (MLGA). Secondly, a broth-based most probable number (MPN) technique, namely Colilert QuantiTray™.

The recovery of coliform organisms from treated water using MLGA was found in some laboratories to be at least equivalent to that of the UK reference medium described in Report 71[3], namely, membrane lauryl sulphate broth (MLSB). For several laboratories, recovery was significantly better. Recovery of coliform organisms from raw waters was geographically variable. The detection of *E. coli* via the detection of ß-glucuronidase, using the substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (BCIG) was markedly more specific than incubation on MLSB at 44°C for treated waters, but specificity varied between laboratories for raw waters. There were insufficient data for comparing the sensitivity in detecting *E. coli* from treated waters, except in one laboratory where results were comparable for MLGA and MLSB. MLGA was significantly less sensitive at detecting *E. coli* in raw waters than MLSB in most laboratories.

Colilert QuantiTray™, being based on the detection of ß-galactosidase, using the substrate o-nitrophenyl-ß-D-galactopyranoside (ONPG), detected more coliform organisms from a range of water types than did MF using MLSB. While significance values have been quoted for each laboratory for the differences between Colilert QuantiTray™ and MF, the number of samples showing differences was sometimes small. Despite using Yates correction (for small samples) when calculating p values, it may be more prudent to conclude, in some cases, that there were insufficient data to draw conclusions as to the significance of the differences. Specificity for *E. coli* was achieved via the detection of ß-glucuronidase using the substrate 4-methylumbelliferyl-ß-D-glucuronide (MUG).

Both MLGA and Colilert QuantiTray™ demonstrated the benefits of enzyme-specific substrates for the detection of *E. coli* and coliform organisms. The use of such substrates in appropriately devised media can allow high reliability in confirmed counts for *E. coli* and coliform organisms within 18 hours.

The study also demonstrated a high geographical variability in the performance of the media and also in the methods being assessed. Results in different laboratories were often significantly at variance, negating any overall conclusions for several aspects of the trial. This may be due to matrix effects or variations in laboratory practices. The results of the study underline the need for individual laboratories to assess the performance of new procedures used within their own laboratories, especially before adopting them on a routine basis.

## 2 Introduction

Established methods, such as described in Report 71[1,3], for the detection and enumeration of *E. coli* and coliform organisms from potable water enable the presumptive isolation of these organisms to be assessed. Subsequent confirmation tests assure the exclusion of false positives being reported. In recent times, MF has been the method of choice in most laboratories processing large numbers of samples. It is usual practice to filter two separate 100 ml volumes of samples and incubate at 44°C and 37°C (for *E. coli* and coliform organisms respectively) in order to give an early indication of the sanitary significance of any isolates encountered.

The detection of *E. coli* and coliform organisms is based on the ability of these organisms to ferment lactose. Fermentation is detected by the incorporation of a pH indicator in the medium (MLSB) which changes colour (ie turns yellow) when acid is produced during fermentation. All colonies that appear yellow or colourless when growing on this medium

are termed "presumptive *E. coli*" or "presumptive coliform organisms", depending on the temperature at which they were incubated. Subsequent tests including production of acid from lactose at 37°C and 44°C, indole from tryptophan at 44°C, Gram reaction and production of cytochrome oxidase are all used to determine whether the organisms are *E. coli* or coliform organisms.

The production of gas from lactose has long been considered not to be a satisfactory diagnostic feature for coliform organisms. This criterion was recognised in the recently published sixth edition of Report 71[3] in its definitions of *E. coli* and coliform organisms. The production of indole from tryptophan at 44°C is not exclusive to *E. coli*. Some strains of *Klebsiella*, notably *K. oxytoca*, are known to produce a positive reaction in this test. In addition, other strains of *E. coli* are indole-negative. Furthermore, not all strains of *E. coli* have the ability to grow at 44°C. One such organism is *E. coli* O157:H7.

With respect to coliform organisms, the currently available tests are even more confusing. Although a significant proportion of coliform organisms is anaerogenic (ie fail to produce gas when fermenting lactose) a notable proportion of coliform organisms isolated from potable sources does not ferment lactose[9]. This is reported as being probably owing to the lack of the enzyme lactose permease. The inability to ferment lactose means that, under the definition outlined in the fifth edition of Report 71[1], these organisms would not be classified as coliform organisms. However, it is perhaps, unreasonable to exclude organisms on the basis of a single physiological test which requires a suite of enzymes for full expression. There seems little logic in assuming that a coliform organism such as a strain of *Enterobacter cloacae* that possesses the enzyme lactose permease is of any more health significance than a strain that does not possess the enzyme.

For public health protection, there is a pressing need to reduce the time interval between taking a sample for examination and the reporting of its results. Recent work has attempted to address this issue via the incorporation of specific diagnostic enzyme tests into media. Suggestions to re-define target organisms of *E. coli* and coliform organisms on the basis of possession of the characterising enzymes of ß-glucuronidase for *E. coli* and ß-galactosidase for coliform organisms have been made over recent years[10,11]. This need for improved definitions for coliform organisms was partly addressed in "Guidance on Safeguarding the Quality of Potable Water Supplies"[2], which was published when the Water Supply (Water Quality) Regulations 1989 (SI 1989/1147) came into force. A key diagnostic feature of the guidance was the expression "normally possessing ß-galactosidase" used in the definition of total coliform organisms. While this approach does not entirely solve the problem over the definition of coliform bacteria as a group, it does allow for the development of simplified tests required to recover and identify desired organisms. For *E. coli*, such tests have now been developed employing fluorogenic or chromogenic substrates, such as 4-methyl-umbelliferyl-ß-D-glucuronide (MUG) or 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (BCIG) for detecting ß-glucuronidase, an enzyme that has been shown to be highly specific to this organism[8].

In 1992, Sartory and Howard[13,14] reported the use of an agar medium based on MLSB incorporating BCIG. This medium, referred to as MLGA, enables the simultaneous enumeration of presumptive coliform organisms and *E. coli* to be determined using a single membrane filter. The medium was evaluated in several laboratories performing bacteriological tests on potable waters and found to perform similarly to MLSB. Presumptive non-*E. coli* coliform organisms appear as yellow colonies owing to the fermentation of lactose. *E. coli* organisms appear as green colonies owing to the combination of the yellow colour as above together with insoluble blue 5,5'-bromo-4,4'-chloro-bisindigo. Occasionally, *E. coli* may appear as blue colonies. This is consistent with the observation that some strains are lactose-negative. Some *Shigella* and *Salmonella* species react similarly to *E. coli* and have been demonstrated to be ß-glucuronidase-positive. This, however, is not considered to be a problem as these organisms are of health significance in their own right. Sartory and Howard[13] identified certain *Aeromonas* species, namely *A. hydrophila* and *A. caviae* from surface waters as other glucuronidase-positive bacteria. Of all the *E. coli* isolated, only 3.8 per cent were ß-glucuronidase-negative. The presence of *E. coli* could be predicted more accurately using MLGA rather than MLSB by counting and picking green colonies. For MLSB, there is no way to discriminate between *E. coli* and thermotolerant coliforms since both produce yellow colonies. The confirmation rates for *E. coli* from MLGA and MLSB were 98 per cent and 70 per cent respectively.

The medium as used and described by Sartory and Howard was noted to be less reliable for relatively polluted waters. It was reported that the expression of ß-glucuronidase may have been inhibited because of overcrowding, and the presence of increased concentrations of lactose catabolites.

Defined substrate technologies[6] have been utilised for the detection of *E. coli* and coliform organisms in water in the United States. These have become widely adopted, primarily as commercially available products. Initial studies with Colilert™[5] showed that similar recoveries to the UK reference method, as described in Report 71[1,3], were obtained for the detection of coliform organisms and *E. coli* in many types of water samples. Following further developments with similar products, extensive studies have now been reported[12]. These results have demonstrated that within a particular geographical area, similar results to the use of duplicate membrane filters can be obtained.

In order to evaluate the performance of MLGA more widely, an inter-laboratory trial was proposed in 1993 under the auspices of the Standing Committee of Analysts. A detailed trial protocol was drawn up against a background of debate over criteria for demonstrating comparability between methods as required by Report 71[3]. In practice, this has usually been interpreted as necessitating an inter-laboratory trial involving at least five independent laboratories so that geographical and "laboratory" variations can be taken into account. For potable waters where most samples are expected to be negative, inter-laboratory evaluations require large numbers of samples to be examined in order to obtain appropriate data for the necessary level of statistical power.

At the time of the evaluations, discussions were also taking place to formalise a change in the definition of "coliform organisms" from a methodological basis to a more genetically based definition, namely the expression of ß-galactosidase activity. As a result, the trial protocol enabled the evaluation of comparative data against both definitions to be assessed.

An inter-laboratory trial was also initiated in 1995 to determine the effectiveness of the QuantiTray™ system, together with Colilert 18™, for the detection and enumeration of coliform organisms and *E. coli* in water. In addition, since it has been suggested[4] that Colilert™ may not be as sensitive as MF for the detection of *E. coli*, experiments were performed with pure cultures of *E. coli* (which had been nutrient stressed in water for 24 hours before dilution) to obtain a theoretical level of one organism per 100 ml. These samples were then used to compare MF with Colilert™.

The two methods, MLGA and QuantiTray™, were compared directly for *E. coli* even though the reference method of Report 71[1,3] effectively amounts to testing twice the volume of water.

## 3 Objectives

The objectives of the trials were to:

(a) assess the performance of MLGA for the single membrane enumeration of *E. coli* and coliform organisms from potable water;

(b) assess the performance of the Colilert 18 QuantiTray™ system for the detection and enumeration of coliform organisms and *E. coli* in water samples analysed routinely in water utility laboratories.

Both trial protocols were intended to determine the relative sensitivity and specificity of the test methods and to compare them with the UK reference MF method employing MLSB based on two membrane, dual-incubation temperature[1,3].

# 4 Materials and methods

4.1 **Laboratories.** Both trials were set up on the basis that a minimum of five laboratories (where the reference method was in routine use) would test raw and non-raw water samples. In the context of this trial, "non-raw" is a contrived term referring to samples of water which were treated or partially treated. The MLGA evaluation was conducted from May to November 1993 and the Colilert QuantiTray ™ trial from June 1995 to March 1996.

4.2 **Water samples.** For each trial, each laboratory set out with the initial objective of examining 100 raw waters and 2000 non-raw waters. Raw water is defined as water abstracted without treatment. Non-raw water samples consisted almost entirely of fully treated drinking water, either at source or at some point within the distribution system.

A sample size of 300 ml was considered as the minimum volume necessary to carry out the comparative exercise; most laboratories routinely took 500 ml samples for drinking water monitoring.

Raw waters were sampled from either boreholes, exposed springs or surface water sources. Laboratories were requested to be selective in their choice of samples and to choose sites where samples were more likely to contain *E. coli* and which were expected to record presumptive coliform counts in the range greater than zero and less than five up to about 80 colonies per 100 ml.

Non-raw water samples, which formed the majority of samples processed, were targeted to sample types where a high proportion of presumptive coliform-positive samples had been experienced in the past. For example, samples from new mains, burst mains, re-samples, incidents, service reservoirs and selected points in distribution systems. However, to avoid introducing bias, participants were advised not to include a disproportionate number of samples from a single incident. Samples from private water supplies (treated or untreated) were also included. In addition, a small proportion of partially treated samples was included. For example, samples of water after slow sand filtration were used to increase the likelihood of obtaining samples containing an appropriate number of presumptive organisms.

For non-raw water samples, an overall failure rate, in terms of samples containing coliform organisms, of 2-3 per cent was anticipated. This figure was deemed sufficient to yield enough data for a meaningful statistical comparison to be made.

4.3 **MLGA evaluation.** A single (28 g) batch of BCIG was purchased for use by participating laboratories undertaking the trial. The substrate was challenge tested before being divided into portions for distribution to participating laboratories. The challenge test consisted of a comparison of response (growth and enzyme expression) of three reference organisms: namely, *E. coli, Shigella sonnei* and *Klebsiella oxytoca*. These organisms were streaked out onto MLGA plates, containing a reference batch of BCIG, and onto plates containing the batch of the chromogen to be used. Challenge testing also included MF of diluted cell suspensions onto test and reference plates.

All other materials used were each supplied by individual participating laboratories.

4.3.1 **Media and procedures.** Routine media, including MLSB, lactose peptone water (LPW), tryptone water (TW), MacConkey agar (MA) and yeast extract agar (YEA) were prepared as described in Report 71[1,3] or else validated alternative commercially available media were used.



MLGA was prepared according to Sartory and Howard[13], namely:

| | |
|---|---|
| MLSB | 76.2 g/l |
| sodium pyruvate | 0.5 g/l |
| BCIG, monocyclohexylammonium salt | 0.2 g/l |
| bacteriological agar | 10.0 g/l |

All ingredients, except BCIG, were mixed and brought to the boil. For a one litre batch, BCIG was prepared by dissolving 200 mg in a combined solution comprising 2.5 ml of 95% aqueous ethanol and 0.5 ml of 1M sodium hydroxide solution before addition to the MLSB medium containing agar. The complete medium was then sterilized by autoclaving at 121°C for 15 minutes. The sterile medium was tempered to 45 - 50°C before pouring into 50 mm petri dishes in volumes of approximately 7 ml. Once set, the plates were inverted and stored refrigerated at 5°C in the dark. Participating laboratories were advised to use the medium as fresh as possible and in any event within 14 days. In general, plates were used within seven days of preparation. Plates were brought to room temperature by standing for 60 to 90 minutes before use.

4.3.1.1 **Sample preparation.** All samples were thoroughly mixed (by rapidly inverting the bottle several times) before filtering.

4.3.1.2 **Membrane filtration.** Three volumes of 100 ml of sample were membrane filtered. Two membrane filters were transferred to plates with pads soaked in MLSB and one to a plate containing MLGA. To avoid bias, sub-samples for the three plates were taken in random order, using a protocol for a sequence of 10 samples derived from random number tables.

The MLGA plate and one MLSB plate were incubated at 30°C for 4.0 ± 0.5 hours followed by 37.0 ± 0.5°C for 14 hours. The other MLSB plate was incubated at 30°C for 4.0 ± 0.5 hours followed by 44.0 ± 0.5°C for 14 hours. The MLSB and MLGA plates for the same sample were incubated in close proximity, and stacking of plates was avoided wherever possible.

4.3.1.3 **Presumptive counts.** Each participating laboratory followed in-house procedures for early reading and re-incubation of MLSB plates. After incubation for the full 18 hours, plates were selected at random and any colonies counted. Where applicable, colony counts were recorded on the back of each plate so as to avoid compromising counts for one medium, by fore-knowledge of the count from another plate. To minimise the variability of counting, it was advised that the same person counted all colonies on both types of plate.

All yellow colonies on MLSB at 37°C were counted as presumptive coliform organisms and at 44°C as presumptive *E. coli*.

All yellow colonies on MLGA were counted as presumptive non-*E. coli* coliform organisms (ie lactose-positive, ß-glucuronidase-negative) and green colonies as presumptive *E. coli* (ie lactose-positive, ß-glucuronidase-positive). The presumptive total coliform count was the sum of yellow and green colonies. Particular interest was attached to the incidence of any blue colonies (ie lactose-negative, ß-glucuronidase-positive) on MLGA. These were subjected to confirmatory tests including oxidase testing, and identification with API 20E, or equivalent, where possible.

4.3.1.4 **Confirmations.** For samples with low counts (ie less than 10), all presumptive colony organisms were picked whenever possible. Each colony was tested for acid and gas production from lactose at 37°C and 44°C, indole from

tryptophan at 44°C and cytochrome oxidase reaction. Oxidase tests were performed from growth obtained from subcultures on YEA or nutrient agar plates.

For higher counts, at least 10 colonies of each presumptive category were picked. For MLSB, 10 presumptive coliform colonies at 37°C and 10 presumptive *E. coli* at 44°C were chosen. For MLGA, 10 yellow or green colonies and additional green colonies to make the total of green colonies up to at least 10 were picked. In practice, this gave a maximum of 40 colonies tested per sample. Colony picking was carried out by clearing a randomly selected area or segment of the plate taking adjacent colonies regardless of size. Using gridded membranes, oriented to give horizontal and vertical lines, this gave 4 possible orientations for counting. Emphasis was placed on randomisation and ensuring that central as well as peripheral areas of the plate were included.

Identification of isolates of presumptive total coliforms subjected to confirmation were recorded according to the following criteria:

MLSB 37°C isolates

Definition A - traditionally confirmed coliform organisms isolated on MLSB at 37°C as defined in Report 71[1] as those that produce acid and gas from LPW at 37°C and are oxidase-negative.

Definition B - traditionally confirmed coliform organisms as defined in Report 71[3] as those that produce acid from LPW at 37°C and are oxidase-negative.

Definition C - isolates obtained at 37CC and confirmed as *E. coli* by conventional tests (acid and gas from LPW, and indole from TW at 44°C and oxidase-negative).

Definition D - isolates obtained at 37°C confirmed as *E. coli* after additional tests such as API 20E.

The number recorded for definition D was either the same as for definition C, or the number recorded for definition C plus the number identified as *E. coli* after additional tests.

MLSB 44°C isolates

*E. coli* number of tested colonies which confirmed as *E. coli* as for definition C and definition D above.

All yellow and green colonies are presumptive coliform organisms. Therefore, colonies that were a mixture of yellow and green colours were picked for total coliform organism confirmation. The colony colour was recorded so that the data for any green colonies picked at the total coliform organism stage could also be used for *E. coli* data evaluation.

When calculating the final confirmed results for each definition per 100 ml, the presumptive count was multiplied by the proportion confirming and rounded to the nearest whole number. For coliform organisms, the exception to this was identified as the circumstance where the presumptive count obtained was higher at 44°C than at 37°C. In this event, the higher of the two counts was quoted. For *E. coli* from MLSB, separate final counts were quoted for data derived from the two temperatures. The same rules were applied to data for MLGA, the final results for *E. coli* being the total number confirming regardless of original colony colour.



The format of the data forms completed by the participating laboratories was designed to facilitate entry onto the database. Participating laboratories were responsible for entering their own data on the database. The data was double-entered for verification purposes, and once completed, a copy (on disc) was sent by each laboratory for subsequent data analysis.

4.3.1.5 **Quality control.** Participating laboratories were requested to record information on media pH after autoclaving, on manufacturer and lot or batch number of media used, and other relevant details of all main media components. Positive and negative growth and/or response tests were performed where appropriate (for example, for confirmation tests).

4.4 **Colilert QuantiTray™ evaluation.** All materials for the QuantiTray™ evaluation were donated by the manufacturer (IDEXX, Chalfont St Peter). The medium (Colilert 18™) was supplied in single aliquot blister packs which contained sufficient medium to be added to a single 100 ml volume of water sample. Sterile sample bottles, containing sodium thiosulphate and an anti-foam reagent, were also supplied. These were used to mix the medium with the 100 ml of water sample. QuantiTray™ packs consist of single use 51-well trays sufficient to hold up to 105 ml of sample. These were supplied gamma irradiated. Quality control data for each component was obtained from the manufacturer. Briefly, this was sterility data for the bottles and packs, and performance data for the medium. The medium was checked with strains of *E. coli*, coliform organisms and *Aeromonas* spp. Each participating laboratory was asked to use the same quality control checks for each batch as they would have normally used for MF.

Water samples (100 ml) were poured into sterile plastic bottles and the medium from the Colilert 18™ sachet was added and the bottles sealed. After vigorous shaking (to dissolve the medium), the mixture was poured into the QuantiTray™ sachet and heat sealed. The QuantiTray™ sachets were then incubated at 37°C for 18 hours. After incubation, the number of wells that showed a yellow colour was recorded. The sachets were then exposed to ultraviolet light irradiation at 365 nm wavelength. The number of wells showing a bright fluorescence was noted. After reference to an MPN table, the number of *E. coli* and coliform organisms was recorded. In addition, all samples were analysed using the two membrane filtration method with MLSB[3] as described above for the MLGA evaluation. Isolates obtained by culture on MLSB were subjected to confirmation tests as described for the MLGA evaluation. Isolates from Colilert QuantiTray™ were not subjected to confirmation tests. The system allows results to be reported on the basis of the possession of and ability to grow utilising one of two specific substrates.

## 5 Statistical methods

5.1 **MLGA evaluation.** Results were entered and checked, using double-entry verification, and data summaries were prepared using the software Epi-Info[7]. Presumptive and confirmed counts fell into wide ranges and could not be transformed to Normal distribution; therefore non-parametric analyses were used. The raw water counts were analysed using the sign test for medians of differenced counts as in the MINITAB software[15].

Example of calculating exact binomial probability:

If counts were small, then the exact binomial probability was calculated. For example, if there were ten samples and five of them gave the same count (including zero counts) by both methods, and four of the remaining counts were higher by one method, and one sample was higher by the other method, then the exact binomial probability was calculated using the null hypothesis assumption that there is a probability of 0.5 that a single result is

higher by one method. (This is similar to a coin being tossed with the expectation of giving heads with a probability of 0.5). Thus, the probability that method A is higher 4 times out of 5 is:

probability (5 times out of 5) + probability (4 times out of 5) =

$$(0.5)^5 + {}^5C_1(0.5)^5 =$$

$$0.03125 + 0.15625 = 0.1875.$$

The two-tailed probability is twice this value (ie 0.375).

The non-raw results were compared for proportions positive (ie proportion of samples where the count was 1 or more). Paired comparisons were tabulated, and discrepant results were tested for similarity (ie numbers of samples which were positive by MLSB and negative by MLGA compared with numbers positive by MLGA and negative by MLSB). Under the null hypothesis of similarity between methods, only chance differences in numbers of positive samples by one method and negative by the other method should be observed. McNemar's test was sometimes used to test this null hypothesis but some numbers were small and so exact binomial probability was used, but instead of "higher" counts being the focus of the comparison, it is discrepancies where a sample gives a positive result by one method but a negative (zero count) by the other method. Two-tailed probabilities were calculated as twice the one-tailed probability obtained using the binomial probability calculation function in GLIM software[16].

5.2 **Colilert QuantiTray™ evaluation.** For comparison of data obtained by Colilert QuantiTray™ and MF using MLSB, McNemar's test for paired samples was used to determine whether one method gave more positive results than the other. Where there were sufficient quantitative data, these were tested using the Wilcoxon matched pairs signed ranks test[17].

## 6 Results

6.1 **MLGA evaluation.** Results from eight laboratories were collected. A total of 14,340 water samples were examined of which 812 were found to contain presumptive coliform organisms by either method. The 812 presumptively positive samples comprised 311 raw water, 478 non-raw and 23 unclassified samples (see Table 1). Water sources are categorised in Table 2. There was considerable variation between laboratories, as would be expected for geographical reasons.

6.1.1 **Non-raw water samples.** Table 3 shows laboratory by source classification for the 478 non-raw water samples that were presumptively positive by at least one method. Of the 478 positive samples, 207 samples were positive by both methods, 202 samples were positive by MLGA only and 69 samples were positive by MLSB only. Thus, 409 samples were presumptively positive by MLGA and 276 samples were presumptively positive by MLSB.

The numbers and proportions of coliform organism-positive samples for both presumptive and confirmed results are presented in Table 4. The probabilities corresponding to the null hypothesis of similarity in performance between the two methods are shown in the square brackets. Results from laboratories 10 and 11 have been combined as numbers of positive samples were small. Both laboratories are part of the same water company and tended to report similar results.

6.1.1.1 **Total coliform organisms.** The proportion of samples found positive by MLGA was higher overall than by MLSB, but there were differences between laboratories. For laboratories 10 and 11, the proportion presumptively positive was significantly higher with MLSB, in contrast to other laboratories. Many of the colonies on MLSB from laboratories 10 and 11 failed to confirm and the differences between methods became insignificant, although numbers were small. Laboratory 20 found significantly more presumptively positive samples by MLGA than by MLSB, although many of the MLGA and some of the MLSB colonies failed to confirm. The differences between methods for confirmed coliform organisms by definitions A and B were reduced and not statistically significant. Laboratory 30, which found the highest number of presumptively positive samples, found a highly significantly larger proportion positive with MLGA. Large numbers of colonies failed to confirm and the differences between methods for confirmed coliform organisms were insignificant. Laboratories 40, 50 and 80 found significantly higher proportions positive by MLGA for both presumptive and confirmed counts. Laboratory 70 showed no difference between methods.

Confirmation rates for definition A and definition B were highly variable between the laboratories (Table 5) for both MLSB and MLGA exemplifying the impact of geographical and laboratory variations. Confirmation rates for the two media varied from 19 per cent to 98 per cent, with the rate increasing by approximately 15 per cent when definition B was applied. For six laboratories, the confirmation rates were comparable for the two media, but for laboratories 30 and 80 confirmation rates were significantly higher on MLSB ($p = <0.001$). The increase in confirmed coliform organisms when definition B was applied resulted in an overall increase in confirmed coliform organism-positive samples from 1.3 per cent and 1.9 per cent to 1.7 per cent and 2.2 per cent respectively for MLSB and MLGA (Table 4).

In summary, MLGA was never less sensitive than MLSB for detecting confirmed coliform organisms. The specificity, however, was significantly worse in two laboratories. Differences between laboratories was the most marked observation and may reflect geographical variation or laboratory performances .

6.1.1.2 ***Escherichia coli.*** The numbers of non-raw water samples containing *E. coli* were too small to draw conclusions. Seven of the eight laboratories found fewer than 10 samples positive. Only Laboratory 80 reported significant numbers of *E. coli*-positive samples and its results show similar numbers positive by both methods. It is, however, not possible to extrapolate this finding to other laboratories because of the variation between laboratories previously commented upon.

One of the key advantages claimed for MLGA is an increased confidence in presumptive identification of *E. coli* [13]. Confirmation rates for the participating laboratories using the traditional definition (definition C) of *E. coli* and formally identified *E. coli* (definition D, which would include anaerogenic and other atypical strains) are given in Table 5. Again the numbers of colonies tested in some laboratories were too few to allow conclusive analysis. Three laboratories tested more than 30 presumptive *E. coli* colonies. Laboratory 40 reported 100 per cent conventional confirmation of isolates from both media. Laboratories 30 and 80 reported significantly higher confirmation rates for MLGA isolates than for those from MLSB ($p = <0.001$), indicating better specificity for MLGA. Most laboratories found no atypical colonies (ie *E. coli* that were not either blue or green) but two laboratories found small numbers. Laboratory 30 reported atypical colonies from MLSB only, and laboratory 80 reported fairly similar numbers from both media. Of the total 156 isolates of presumptive *E. coli* from MLGA from all laboratories, 152 (97.4 per cent) were identified as *E. coli* (as defined by definition D) compared to 70.5 per cent (ie 177 from 251 isolates) from MLSB.

6.1.2 **Raw water samples.** Table 6 shows laboratory by source classification for the 311 presumptive coliform-positive samples.

A summary of counts per 100 ml of sample for the two methods by laboratory is given in Table 7. The number of presumptively positive samples for each laboratory is shown in the first column; subsequent columns give the median and range of counts for those particular samples. The overall medians are shown in the final row and were below 10 per 100 ml, although individual counts of up to 18000 were recorded. The overall medians appear similar for MLSB compared with MLGA, but this misrepresents the findings within individual laboratories.

Tables 8 and 9 show the statistical comparisons of these median counts between the two methods. Table 8 gives results for total coliform organisms and Table 9 for *E. coli* counts.

6.1.2.1 **Total coliform organisms.** Within laboratories, the results for MLGA appeared to give similar or slightly lower average counts to MLSB. There was some variation between laboratories and between definitions.

Laboratory 10 tended to report a greater number of higher presumptive counts with MLSB ($p = 0.08$, Table 8) and significantly greater number of higher confirmed counts on MLSB by either definition ($p = 0.016$, $p = 0.028$). Laboratory 11 also had significantly higher counts from MLSB for presumptive and both confirmed counts ($p = 0.18$, $p = 0.02$, $p = 0.02$). Laboratories 20, 30 and 40 found similar confirmed counts by MLSB and MLGA. Laboratory 70 reported significantly higher presumptive total coliform counts by MLGA ($p = 0.02$); the difference appeared to hold for confirmed counts by definition B ($p = 0.052$) but not by definition A ($p = >0.4$). Laboratory 80 reported more samples with higher confirmed counts by MLSB, but the difference between methods was not significant.

It is not possible to give a single, realistic estimate of the difference between MLSB and MLGA average counts with a 95 per cent confidence interval because of the variation between laboratories. The strongest statistical finding was the interaction between laboratory and method.

6.1.2.2 ***Escherichia coli.*** Within laboratories, the results for MLGA appeared to give lower average *E. coli* counts than MLSB. There was some variation between laboratories. Laboratory 40 reported few presumptively positive samples and no significant difference could be detected. Laboratory 80 found higher presumptive counts by MLSB from most samples ($p = <0.00001$) but the difference was not so marked for confirmed counts ($p = 0.03$). In all other laboratories, the presumptive and confirmed *E. coli* counts tended to be higher with MLSB.

The rates of confirmation of coliform organisms by definitions A and B and of *E. coli* by definitions C and D are given in Table 10. Five laboratories reported similar rates for confirming coliform organisms by definitions A and B for both MLGA and MLSB, but in laboratories 40 and 70, significantly more colonies from MLSB confirmed as coliform organisms for both definitions than did from MLGA. For *E. coli*, the proportion of presumptive colonies which confirmed by either definition C or D were similar for the two media in five laboratories. In laboratory 40, there was a highly significant increased confirmation rate for MLSB ($p = 0.0006$), while in laboratory 80, there was a significant increased confirmation rate for MLGA ($p = <0.0001$). This, again, emphasises the impact of geographical variation.

## 6.2 Colilert QuantiTray™ evaluation

6.2.1 **Total coliform organisms.** Of the 10,516 samples examined, 309 (2.9 per cent) samples were shown to contain coliform organisms by at least one method. Colilert QuantiTray™ detected coliform organisms in 273 samples of the 309 (88.3 per cent) samples found to be positive, while MF detected coliform organisms in 264 (85.4 per cent) samples of the positive samples (Table 11). Of the 309 samples that were positive for coliform organisms, 228 samples were positive by both methods while 45 samples were positive by Colilert QuantiTray™ only, and 36 samples by MF only. When examined by McNemar's test for paired samples, these differences are not significant ($p = >0.05$). When examining the quantitative data, samples that were reported as "greater than" were excluded. Table 13 shows that Colilert QuantiTray™ detected a total of 5983 coliform organisms, whereas MF detected only 3429 (data from CLAB 1 were excluded as these were from *E. coli*-positive AQC samples). Table 11 shows that for seven laboratories, Colilert QuantiTray™ found more samples to be positive while the converse was true for the remaining two laboratories. Notwithstanding this, one laboratory (CLAB 2) reported Colilert QuantiTray™ to give significantly more samples positive for coliform organisms, while one (CLAB 10) reported significantly more samples positive by MF using MLSB. All other laboratories reported no significant difference in the number of samples found to contain coliform organisms using the two methods. All laboratories reported a higher total number of coliform organisms being detected by Colilert QuantiTray™ than by MF (Table 13).

Of the 50 quality control samples that were examined, five gave the same results by MF (using MLSB) at 37°C and QuantiTray™, 12 gave a higher result with MLSB, and 33 QC samples gave a higher result with QuantiTray™.

6.2.2 ***Escherichia coli.*** A total of 200 (1.9 per cent) of 10,516 water samples were found to contain *E. coli* by at least one method. Colilert QuantiTray™ detected *E. coli* in 167 (83.5 per cent) of the samples found to be positive, whereas MLSB detected *E. coli* in 188 (94.0 per cent) samples, see Table 12. Of the 200 samples containing *E. coli*, 153 samples were positive by both methods, 14 samples were positive by Colilert QuantiTray™ alone, and 33 samples were positive by MF alone. This difference is significant by McNemar's test ($p = <0.01$), although this is heavily influenced by the data from laboratory CLAB 10. When examining the quantitative data, samples that were reported as "greater than" were excluded. Colilert QuantiTray™ detected a total of 3100 *E. coli* organisms while MF detected 2737, see Table 13. (Data from CLAB 1 were excluded as these results were all from *E. coli*-positive AQC samples). In one laboratory, CLAB 10, MF was shown to detect *E. coli* in significantly more samples than Colilert QuantiTray™, one laboratory (CLAB 2) found significantly more samples positive by Colilert QuantiTray™, while for the remaining seven laboratories that detected *E. coli*, no significant difference was seen (Table 12). Six laboratories reported detecting more total numbers of *E. coli* with MF (one significantly), one laboratory detected exactly the same number of organisms with both methods, and two laboratories detected more *E. coli* organisms with Colilert QuantiTray™ (one significantly) (see Table 13).

One laboratory provided data for 50 quality control samples. The organism used was an environmental strain of *E. coli* and this was enumerated using MF incubated on MLSB at 37°C and 44°C and using Colilert QuantiTray™. On eight occasions, the 44°C MLSB membrane gave higher results than Colilert QuantiTray™, and on 42 occasions, Colilert QuantiTray™ gave higher counts.

The paired data from *E. coli*-positive samples from all laboratories, except CLAB 1, were tested for significant differences by Wilcoxon matched-pairs signed ranks test. Only laboratories CLAB 6 and CLAB 10 had sufficient data for independent analysis. The data from the remaining seven laboratories were pooled. For the pooled laboratories' data, there was no significant difference in counts from MLSB and Colilert QuantiTray™ ($n = 50$, $p = >0.1$). For laboratory CLAB 6, counts from Colilert QuantiTray™ were significantly greater ($n = 37$, $p = <0.01$), while at laboratory CLAB 10, counts from MLSB were

significantly greater (n = 102, p = <0.001). When the total number of samples (from all laboratories) which were found to contain *E.coli* was examined, MF detected *E.coli* in significantly more samples than was reported by Colilert QuantiTray™. However, these differences were due entirely to the results reported by one laboratory (CLAB 10). When the data from this laboratory were excluded, the number of samples reported to contain *E.coli* (from all laboratories) was the same for both methods. Thus, while it is clear that MF performed significantly better than Colilert QuantiTray™ in one laboratory, generally, there was similarity between the two methods, although numbers were small.

# 7 Discussion

In common with other water methodology trials, the most prominent observation with both trials was the differences between laboratories (ie in statistical terminology the interaction between laboratory and methods). This means that an overall conclusion about the new methods cannot be stated simply, as every remark has to be qualified for geographical variation.

It has been suggested that validation trials of methods to be used with samples expected to be negative should be carried out with spiked samples. Such samples can be drawn from a batch to provide large numbers of positive samples which will give the required amount of statistical "information". However, with indicator organisms, the "real life" sample approach is supported by both the MLGA and Colilert QuantiTray™ trials. Although initially this seems inefficient because of the large numbers of samples that need to be examined, the "real life" non-raw water samples clearly give a different picture to the raw samples.

With *E. coli*, because of the small numbers of samples that were positive, it is difficult to draw conclusions based on statistics, particularly for non-raw waters. Both trials, however, tested large numbers of samples and if there were gross differences in the ability to detect *E. coli*, then this should have been apparent despite the limited number of positive samples. Individual laboratories may need to augment this work with their own supplementary evaluation to ensure that they are able to achieve comparable results.

This highlights the practicalities of evaluations requiring large numbers of samples to obtain a sufficient number of positive samples in order to generate an acceptable data base for valid statistical analyses.

7.1 **MLGA evaluation.** The data generated during this evaluation support the approach of utilising a medium containing a specific substrate detection system for the single membrane enumeration of *E. coli* and coliform organisms. Detection of expression of ß-glucuronidase allows for more reliable identification of presumptive *E. coli*, particularly for potable water samples (ie non-raw water samples as depicted in these trials). The medium evaluated in this study, MLGA, appears to be at least comparable to MLSB for the isolation of coliform organisms and, for some laboratories, resulted in increased recoveries. Recoveries for *E. coli* appeared similar on MLGA and MLSB but the data were insufficient for all but one laboratory, and, therefore, the confidence intervals for the estimated recovery rate differences remain wide. Thus, sensitivity of MLGA appears to be comparable to, or better than, MLSB (in all the trial laboratories) for coliform organisms, and comparable for *E. coli*.

Specificity for non-raw water samples varied geographically. Within six laboratories, the confirmation rates for coliform organisms from MLGA and MLSB were similar, while for the remaining two laboratories, they were higher for MLSB. However, for *E. coli*, the confirmation rates for the same two laboratories were higher on MLGA, and appeared similar in the other laboratories, albeit with limited data. The overall confirmation rates from this evaluation are comparable to those reported earlier[13,14].



For raw waters, performance of MLGA in enumerating total coliform organisms seems to vary between laboratories and may or may not be equivalent to that of MLSB. For *E. coli*, however, MLGA performed worse than MLSB in most laboratories. This reduced performance of MLGA for raw waters was reported previously[13,14] where it was suggested that the use of the medium for such waters would necessitate incubation of a second membrane at 44°C. It was suspected that this deterioration in performance with polluted waters containing high populations may be related to crowding effects or lactose catabolite repression[14].

With raw waters, MLGA was less specific for coliform organisms for two laboratories, in that lower rates of organisms were confirmed. Comparable rates were recorded from the other five laboratories. For *E. coli*, the specificity was more variable between laboratories and MLGA showed better specificity than MLSB at one laboratory, but worse at another. Poor specificity for *E. coli* from raw waters may be related to the presence of some strains of *Aeromonas* that are ß-glucuronidase-positive[14].

In this study, sodium pyruvate was incorporated at 0.05%[13] on the basis that it may improve the recovery of environmentally or disinfectant-stressed coliform organisms. Comparing the data for total coliform organisms from the two media appears tentatively to support this hypothesis.

7.2 **Colilert QuantiTray™ evaluation.** It is clear from the data obtained that Colilert QuantiTray™ recovered more coliform organisms than MF, both in terms of the number of samples found to be positive and the total number of isolates obtained. Only one laboratory had more samples positive for coliform organisms by MF using MLSB than by Colilert QuantiTray™. This laboratory also had significantly more samples positive for *E. coli* when using MLSB than when using Colilert QuantiTray™. There are many possible explanations for this which include the effect of the particular water type, the phenotypic characteristics of the organisms present within the particular geographical area, and the interaction between the laboratory personnel and any one particular method. Most laboratories showed no significant difference between the two procedures for *E. coli*, both in terms of the number of samples found to be positive and the total number of organisms detected.

7.3 **General.** Some differences were seen between laboratories for both the MLGA and the Colilert QuantiTray™ trials. It is impossible to identify the exact reason for these differences. In a recent report[4] comparing the use of a number of presence/absence media for the detection of coliforms and *E. coli*, it has been suggested that these differences may be caused by matrix effects. Differences between results obtained by participating laboratories, however, may be caused by different practices between laboratories. The factors relating to these differences need to be understood, their impact assessed, and, where possible, minimised.

The use of real samples is to be commended for inter-laboratory trials of new media and methods, despite the fact that these types of microbiological trials are time consuming and labour intensive. It is unlikely that any single method will perform better than a reference method for all samples, particularly in microbiological tests for the detection of *E. coli* and coliform organisms. This is particularly true for these types of tests where, for samples containing low numbers of organisms, differences are often seen by chance. From a statistical point of view, this means challenging the methods with a large number of organisms. In theory, that could be done efficiently with a spiked sample from which large numbers of sub-samples are taken. In practice, however, the microbiological content of real waters is so variable, geographically, that meaningful conclusions are best found with real samples. Spiked samples yield limited information, which cannot be extrapolated beyond the actual organism types and background conditions used for the spiked water.

In the studies reported here, quantitative methods were being compared and thus it is less important to have samples with very low numbers of organisms, since statistical methods can be applied to determine if one method is recovering more organisms than another. This is not the case when comparing presence/absence methods where it is extremely important to use samples with low numbers of organisms, since only one cell is required to give a positive result. This could mean that in a sample containing 100 coliform organisms per ml, only one of the 100 organisms might grow. However, this would still give rise to a positive result. Thus, the qualitative and quantitative methods would give the same result, ie positive. However, when comparing quantitative methods, the actual numbers of micro-organisms as well as the number of samples found to be positive can be compared. Thus, the sensitivity of the reference and test methods can be compared in two ways.

## 8 References


1. 1983. The Bacteriological Examination of Drinking Water Supplies 1982. Methods for the Examination of Water and Associated Materials. Reports on Public Health and Medical Subjects No.71, in this series.

2. 1989. Guidance on Safeguarding the Quality of Public Water Supplies. Department of Environment/Welsh Office. London: HMSO.

3. 1994. The Microbiology of Water 1994. Part 1 - Drinking Water. Methods for the Examination of Waters and Associated Materials. Reports on Public Health and Medical Subjects No.71, in this series.

4. 1996. An Evaluation of Presence-Absence Tests for Coliform Organisms and *Escherichia coli* 1996. Methods for the Examination of Waters and Associated Materials, in this series.

5. COWBURN, J.K., GOODALL, T., FRICKER, E.J., WALTER, K.S. and FRICKER, C.R. 1994. A preliminary study of the use of Colilert for water quality monitoring. *Letters in Applied Microbiology* **19**, 50-52.

6. EDBERG, S.C., ALLEN, M.J. and SMITH, D.B. 1991. Defined substrate technology method for rapid and specific enumeration of total coliforms and *Escherichia coli* from water. Collaborative study. *Journal of the Association of Official Analytical Chemists* **74**, 526-529.

7. DEAN, A.G. 1990. Epi-Info Version 5 Manual. USD Inc., Stone Mountain, Georgia, USA. Epi-Info, version 5, obtained from CDSC, PHLS, Colindale Avenue, London.

8. FRAMPTON, E.W. and RESTAINO, L. 1993. A Review. Methods for *Escherichia coli* identification in food, water and clinical samples based on beta-glucuronidase detection. *Journal of Applied Bacteriology* **74**, 223-233.

9. FRICKER, C.R., COWBURN, J., GOODALL, T., WALTER, K.S. and FRICKER, E.J. 1994. Use of the Colilert system in a large U.K. water utility. *Proceedings of the 1993 Water Quality Technology Conference,* Florida 1993, American Water Works Association, 525-527.

10. FRICKER, E.J. and FRICKER, C.R. 1994. Application of the polymerase chain reaction to the identification of *Escherichia coli* and coliforms in water. *Letters in Applied Microbiology* **19**, 44-46.

11. FRICKER, E.J. and FRICKER, C.R. 1996. Use of two presence/absence systems for the detection of *E. coli* and coliforms from water. *Water Research* **30**, 2226-2228.

12. FRICKER, E.J., ILLINGWORTH, K.S. and FRICKER, C.R., Use of two formulations of Colilert and QuantiTray for assessment of the bacteriological quality of water. Water Research Vol 31 No 11 pp 2495 - 2499.

13. SARTORY, D.P. and HOWARD, L. 1992a. A medium detecting ß-glucuronidase for the simultaneous membrane filtration enumeration of Escherichia coli and coliforms from drinking water. *Letters in Applied Microbiology* **15**, 273-276.

14. SARTORY, D.P and HOWARD, L. 1992b. A simple membrane enumeration medium for coliforms and *E. coli* utilising a chromogenic glucuronide substrate. *Proceedings of the 1992 Water Quality Technology Conference,* Toronto 1992, American Water Works Association, 1661-1673.

15. MINITAB, 1989, release 7, Minitab Inc., State College, PA, USA.

16. GLIM, 1993. Generalised Linear Interactive Modelling Systems, National Algorithms Group, Oxford, UK.

17. STATSGRAPHICS, 1991, version 5, STSC Inc., Rockville, MA, USA.


## 9 Participating laboratories in the evaluation trials

### 9.1 MLGA

South West Water
Wessex Water Services
Strathclyde Regional Council
Acer Environmental
Yorkshire Water Laboratory Services
North West Water
Lothian Regional Council

### 9.2 Colilert QuantiTray™

AES
Southern Science
Wessex Water Services
Thames Water Utilities
Hyder Environmental
GU Projects
Strathclyde Regional Council
North West Water
Anglian Water
Yorkshire Water Laboratory Services

**Table 1** Numbers and types of water samples examined in the evaluation of MLGA

| Laboratory code | Number of raw water samples | Number of non-raw water samples | Number of samples type not known | Total number of samples |
|---|---|---|---|---|
| LAB 10 | 86 (47) | 786 (10) | 45 (4) | 917 (61) |
| LAB 11 | 16 (10) | 765 (14) | 20 (7) | 801 (31) |
| LAB 20 | 77 (65) | 2017 (75) | 16 (10) | 2110 (150) |
| LAB 30 | 50 (49) | 2105 (133) | 1 (1) | 2156 (183) |
| LAB 40 | 29 (12) | 2067 (39) | 2 (0) | 2098 (51) |
| LAB 50 | | 1948 (30) | | 1948 (30) |
| LAB 70 | 28 (28) | 2133 (71) | 24 (1) | 2185 (100) |
| LAB 80 | 100 (100) | 2025 (106) | | 2125 (206) |
| Total | 386 (311) | 13846 (478) | 108 (23) | 14340 (812) |

The figures in brackets represent the number of samples where positive results were obtained.

**Table 2** Classification of all samples in relation to abstraction sources

| Lab. code | Upland surface | Low-land surface | Spring | Ground | Blended | Other | Not known | Total |
|---|---|---|---|---|---|---|---|---|
| LAB 10 | 77 | 418 | 59 | 100 | 209 | 31 | 23 | 917 |
| LAB 11* | 0 | 0 | 1 | 4 | 0 | 780 | 16 | 801 |
| LAB 20 | 0 | 493 | 415 | 860 | 131 | 0 | 211 | 2110 |
| LAB 30 | 2156 | 0 | 0 | 0 | 0 | 0 | 0 | 2156 |
| LAB 40 | 644 | 574 | 173 | 140 | 502 | 6 | 59 | 2098 |
| LAB 50 | 1400 | 545 | 0 | 3 | 0 | 0 | 0 | 1948 |
| LAB 70 | 272 | 265 | 92 | 168 | 1380 | 8 | 0 | 2185 |
| LAB 80 | 1851 | 0 | 260 | 0 | 14 | 0 | 0 | 2125 |

* LAB 11 did not identify the sources of non-raw water samples.

**Table 3** Classification of coliform organism-positive* non-raw water samples in relation to abstraction sources

| Lab. code | Upland surface | Low-land surface | Spring | Ground | Blended | Other | Not known | Total |
|---|---|---|---|---|---|---|---|---|
| LAB 10 | 3 | 4 | 0 | 1 | 2 | 0 | 0 | 10 |
| LAB 11 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 14 |
| LAB 20 | 0 | 20 | 11 | 30 | 6 | 0 | 8 | 75 |
| LAB 30 | 133 | 0 | 0 | 0 | 0 | 0 | 0 | 133 |
| LAB 40 | 8 | 9 | 1 | 5 | 6 | 0 | 10 | 39 |
| LAB 50 | 27 | 3 | 0 | 0 | 0 | 0 | 0 | 30 |
| LAB 70 | 2 | 7 | 1 | 1 | 60 | 0 | 0 | 71 |
| LAB 80 | 87 | 0 | 19 | 0 | 0 | 0 | 0 | 106 |
| Total | 260 | 43 | 32 | 37 | 74 | 14 | 18 | 478 |

* Presumptive coliform organism/*E. coli* recorded by at least one method.



**Table 4** Number (percentage) and [statistical comparison*] of coliform organism-positive samples from non-raw water samples for MLSB and MLGA

| Lab Code (Total number non-raw samples) | Presumptive Coliforms | | Coliforms by Definition A† | | Coliforms by Definition B† | | *E. coli* by Definition C† | |
|---|---|---|---|---|---|---|---|---|
| | MLSB | MLGA | MLSB | MLGA | MLSB | MLGA | MLSB | MLGA |
| LAB 10&11 (1551) | 21 (1.3) | 12 (0.8) [0.03] | 11 (0.7) | 9 (0.6) [>0.3] | 14 (0.9) | 9 (0.6) [0.26] | 5 (0.3) | 3 (0.2) |
| LAB 20 (2017) | 47 (2.3) | 65 (3.2) [0.005] | 36 (1.8) | 47 (2.3) [0.08] | 42 (2.1) | 53 (2.6) [0.11] | 5 (0.2) | 7 (0.3) |
| LAB 30 (2105) | 70 (3.3) | 116 (5.5) [<0.00001] | 38 (1.8) | 42 (2.0) [>0.3] | 54 (2.6) | 60 (2.9) [>0.3] | 9 (0.4) | 5 (0.2) |
| LAB 40 (2067) | 18 (0.9) | 39 (1.9) [<0.00001] | 12 (0.6) | 30 (1.5) [<0.00001] | 12 (0.6) | 31 (1.5) [<0.00001] | 4 (0.2) | 5 (0.2) |
| LAB 50 (1948) | 13 (0.7) | 30 (1.5) [<0.00001] | 12 (0.6) | 25 (1.3) [0.0002] | 12 (0.6) | 27 (1.4) [0.0001] | 0 (-) | 2 (0.1) |
| LAB 70 (2133) | 51 (2.4) | 49 (2.3) [>0.3] | 22 (1.0) | 24 (1.1) [>0.3] | 42 (2.0) | 36 (1.7) [>0.3] | 5 (0.2) | 3 (0.1) |
| LAB 80 (2025) | 56 (2.8) | 98 (4.8) [<0.00001] | 55 (2.7) | 90 (4.4) [0.001] | 56 (2.8) | 92 (4.5) [<0.00001] | 35 (1.7) | 34 (1.7) |
| Total (13846) | 276 | 409 | 186 | 267 | 232 | 308 | 63 | 59 |

*Exact binomial probability, two-tailed
†See text for explanation of definitions.

**Table 5** Confirmation rates for presumptive coliform organisms and *E. coli* isolates from non-raw water samples recovered on MLSB and MLGA

| Laboratory code | MLSB | | | MLGA | | |
|---|---|---|---|---|---|---|
| | Coliform organisms | | | | | |
| | Number of isolates tested | Number confirmed by definition: | | Number of isolates tested | Number confirmed by definition: | |
| | | A† (%) | B† (%) | | A† (%) | B†(%) |
| LAB 10 | 18 | 15 (83) | 16 (89) | 15 | 12 (80) | 14 (93) |
| LAB 11 | 35 | 13 (37) | 30 (86) | 23 | 7 (30) | 8 (35) |
| LAB 20 | 229 | 154 (67) | 174 (76) | 252 | 175 (69) | 205 (81) |
| LAB 30 | 283 | 113 (40) | 171 (60) | 574 | 109 (19) | 216 (38) |
| LAB 40 | 102 | 77 (76) | 77 (76) | 245 | 178 (73) | 183 (75) |
| LAB 50 | 76 | 52 (68) | 65 (86) | 116 | 70 (60) | 104 (90) |
| LAB 70 | 160 | 46 (29) | 110 (69) | 138 | 49 (36) | 94 (68) |
| LAB 80 | 277 | 270 (98) | 270 (98) | 461 | 412 (89) | 422 (92) |

| Laboratory code | *E. coli* | | | | | |
|---|---|---|---|---|---|---|
| | Number of isolates tested | Number confirmed by definition: | | Number of isolates tested | Number confirmed by definition: | |
| | | C† (%) | D† (%) | | C† (%) | D†(%) |
| LAB 10 | 0 | - | - | 2 | 2 (100) | 2 (100) |
| LAB 11 | 2 | 0 (0) | 0 (0) | 2 | 2 (100) | 2 (100) |
| LAB 20 | 6 | 6 (100) | 6 (100) | 7 | 4 (57) | 4 (57) |
| LAB 30 | 40 | 13 (33) | 23 (58) | 13 | 12 (92) | 12 (92) |
| LAB 40 | 22 | 22 (100) | 22 (100) | 12 | 12 (100) | 12 (100) |
| LAB 50 | 2 | 0 (0) | 0 (0) | 0 | - | - |
| LAB 70 | 12 | 7 (58) | 7 (58) | 4 | 4 (100) | 4 (100) |
| LAB 80 | 167 | 105 (63) | 119 (71) | 116 | 105 (91) | 116 (100) |

† See text for explanation of definitions.

**Table 6** Classification of coliform organism-positive* raw water samples in relation to abstraction sources

| Lab. code | Upland surface | Low-land surface | Spring | Ground | Blended | Other | Not known | Total |
|---|---|---|---|---|---|---|---|---|
| LAB 10 | 9 | 4 | 13 | 16 | 5 | 0 | 0 | 47 |
| LAB 11 | 0 | 0 | 0 | 1 | 0 | 9 | 0 | 10 |
| LAB 20 | 0 | 34 | 18 | 11 | 0 | 0 | 2 | 65 |
| LAB 30 | 49 | 0 | 0 | 0 | 0 | 0 | 0 | 49 |
| LAB 40 | 4 | 2 | 4 | 0 | 0 | 0 | 2 | 12 |
| LAB 70 | 6 | 5 | 4 | 4 | 9 | 0 | 0 | 28 |
| LAB 80 | 88 | 0 | 12 | 0 | 0 | 0 | 0 | 100 |
| Totals | 156 | 45 | 51 | 32 | 14 | 9 | 4 | 311 |

* Presumptive coliform organisms/*E. coli* recorded by at least one method.



**Table 7** Median counts and (ranges) per 100 ml from raw water samples

| Lab Code | Presumptive coliforms | | Coliforms by definition A† | | Coliforms by definition B† | | Presumptive *E. coli** | | *E. coli* by definition C† | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MLSB | MLGA | MLSB‡ | MLGA | MLSB‡ | MLGA | MLSB | MLGA | MLSB‡ | MLGA |
| LAB 10 (47) | 4 (1-142) | 4 (1-140) | 4 (1-142) | 4 (1-140) | 4 (1-142) | 4 (1-140) | 1 (0-123) | 0 (0-140) | 2 (0-142) | 0 (0-140) |
| LAB 11 (10) | 29 (1-1000) | 12 (0-3000) | 13 (0-1000) | 12 (0-333) | 26 (0-1000) | 12 (0-333) | 20 (0-2000) | 10 (0-170) | 20 (0-2000) | 10 (0-170) |
| LAB 20 (65) | 10 (0-18000) | 9 (0-11000) | 10 (0-9000) | 7 (0-4400) | 10 (0-12600) | 7 (0-4400) | 2 (0-1500) | 1 (0-1600) | 3 (0-7200) | 2 (0-2200) |
| LAB 30 (49) | 10 (0-250) | 10 (0-225) | 9 (0-250) | 9 (0-255) | 9 (0-250) | 9 (0-255) | 4 (0-250) | 4 (0-56) | 8 (0-250) | 6 (0-255) |
| LAB 40 (12) | 7 (0-6100) | 7 (0-8200) | 7 (0-6100) | 3 (0-8200) | 7 (0-6100) | 3 (0-8200) | 4 (0-2800) | 1.5 (0-1900) | 4.5 (0-6100) | 1 (0-8200) |
| LAB 70 (28) | 45 (0-380) | 60.5 (0-2020) | 40 (0-380) | 36.5 (0-1616) | 40 (0-380) | 56 (1-1818) | 21 (0-250) | 17 (0-350) | 40 (0-266) | 15.5 (0-315) |
| LAB 80 (100) | 8 (0-100) | 8 (0-98) | 8 (0-180) | 8 (0-180) | 8 (0-180) | 8 (0-180) | 6.5 (0-75) | 1 (0-51) | 2 (0-76) | 2 (0-51) |
| Totals (311) | 9.5 (0-18000) | 9 (0-11000) | 9 (0-9000) | 8 (0-8200) | 9 (0-12600) | 8 (0-8200) | 4 (0-2800) | 2 (0-1900) | 4 (0-7200) | 3 (0-8200) |

† See text for explanation of definitions.
* Counts at 44°C for MLSB, green colonies on MLGA.
‡ Based upon the higher of the counts at either 37°C or 44°C.

**Table 8** Comparison of MLSB and MLGA using paired results for total coliform organism counts per 100 ml from raw waters

Presumptive counts

| | MLSB compared with MLGA | | | | |
|---|---|---|---|---|---|
| Lab. code | Lower | Equal | Higher | Total | P value* |
| LAB 10 | 11 | 14 | 22 | 47 | 0.08 |
| LAB 11 | 2 | 1 | 7 | 10 | 0.18 |
| LAB 20 | 30 | 11 | 24 | 65 | > 0.4 |
| LAB 30 | 23 | 2 | 24 | 49 | > 0.4 |
| LAB 40 | 8 | 2 | 2 | 12 | 0.11 |
| LAB 70 | 20 | 1 | 7 | 28 | 0.02 |
| LAB 80 | 38 | 11 | 51 | 100 | 0.2 |
| Totals | 132 | 42 | 137 | 311 | > 0.4 |

Confirmed counts by definition A†

| | MLSB compared with MLGA | | | | |
|---|---|---|---|---|---|
| Lab. Code | Lower | Equal | Higher | Total | P value* |
| LAB 10 | 10 | 12 | 25 | 47 | 0.016 |
| LAB 11 | 1 | 0 | 9 | 10 | 0.02 |
| LAB 20 | 25 | 13 | 27 | 65 | > 0.4 |
| LAB 30 | 19 | 5 | 25 | 49 | > 0.4 |
| LAB 40 | 6 | 3 | 3 | 12 | > 0.4 |
| LAB 70 | 14 | 3 | 11 | 28 | > 0.4 |
| LAB 80 | 37 | 13 | 50 | 100 | 0.2 |
| Totals | 112 | 49 | 150 | 311 | 0.02 |

Confirmed counts by definition B†

| | MLSB compared with MLGA | | | | |
|---|---|---|---|---|---|
| Lab. Code | Lower | Equal | Higher | Total | P value* |
| LAB 10 | 11 | 11 | 25 | 47 | 0.028 |
| LAB 11 | 1 | 0 | 9 | 10 | 0.02 |
| LAB 20 | 28 | 11 | 26 | 65 | > 0.4 |
| LAB 30 | 21 | 3 | 25 | 49 | > 0.4 |
| LAB 40 | 6 | 3 | 3 | 12 | > 0.4 |
| LAB 70 | 19 | 1 | 8 | 28 | 0.052 |
| LAB 80 | 37 | 14 | 49 | 100 | 0.25 |
| Totals | 123 | 43 | 145 | 311 | 0.19 |

† See text for explanation of definitions.
* Exact binomial probability (two-tailed) of discrepant results.



**Table 9** Comparison of MLSB and MLGA using paired results for *E. coli* counts per 100 ml from raw water samples

Presumptive counts

| | MLSB compared with MLGA | | | | |
|---|---|---|---|---|---|
| Lab. code | Lower | Equal | Higher | Total | P value* |
| LAB 10 | 8 | 22 | 17 | 47 | 0.11 |
| LAB 11 | 1 | 3 | 6 | 10 | 0.12 |
| LAB 20 | 11 | 21 | 33 | 65 | 0.0013 |
| LAB 30 | 12 | 9 | 28 | 49 | 0.017 |
| LAB 40 | 2 | 4 | 6 | 12 | 0.28 |
| LAB 70 | 6 | 3 | 19 | 28 | 0.014 |
| LAB 80 | 9 | 16 | 75 | 100 | < 0.00001 |
| Totals | 49 | 78 | 184 | 311 | << 0.00001 |

Confirmed counts by definition C†

| | MLSB compared with MLGA | | | | |
|---|---|---|---|---|---|
| Lab. code | Lower | Equal | Higher | Total | P value* |
| LAB 10 | 9 | 19 | 19 | 47 | 0.08 |
| LAB 11 | 1 | 3 | 6 | 10 | 0.12 |
| LAB 20 | 12 | 19 | 34 | 65 | 0.002 |
| LAB 30 | 14 | 8 | 27 | 49 | 0.06 |
| LAB 40 | 4 | 5 | 3 | 12 | > 0.4 |
| LAB 70 | 4 | 3 | 21 | 28 | 0.001 |
| LAB 80 | 21 | 41 | 38 | 100 | 0.03 |
| Totals | 65 | 98 | 148 | 311 | << 0.00001 |

† See text for explanation of definitions.
* Exact binomial probability (two-tailed) of discrepant results.

**Table 10** Confirmation rates for presumptive coliform organisms and E. coli isolates from raw water samples recovered on MLSB and MLGA

| | MLSB | | | MLGA | | |
|---|---|---|---|---|---|---|
| **Coliform organisms** | | | | | | |
| Laboratory code | Number of isolates tested | Number confirmed by definition: A† (%) | B† (%) | Number of isolates tested | Number confirmed by definition: A† (%) | B†(%) |
| LAB 10 | 252 | 230 (91) | 246 (98) | 264 | 235 (89) | 264 (100) |
| LAB 11 | 38 | 29 (76) | 35 (92) | 29 | 25 (86) | 26 (90) |
| LAB 20 | 379 | 320 (84) | 331 (87) | 380 | 314 (83) | 329 (87) |
| LAB 30 | 309 | 287 (93) | 294 (95) | 348 | 299 (86) | 313 (90) |
| LAB 40 | 59 | 54 (92) | 54 (92) | 64 | 51 (80) | 51 (80) |
| LAB 70 | 236 | 208 (88) | 222 (94) | 254 | 166 (65) | 221 (87) |
| LAB 80 | 651 | 646 (99) | 650 (100) | 679 | 667 (98) | 669 (99) |
| ***E. coli*** | | | | | | |
| Laboratory code | Number of isolates tested | Number confirmed by definition: C† (%) | D† (%) | Number of isolates tested | Number confirmed by definition: C† (%) | D†(%) |
| LAB 10 | 112 | 105 (94) | 105 (94) | 105 | 105 (100) | 105 (100) |
| LAB 11 | 30 | 28 (93) | 28 (93) | 26 | 23 (89) | 23 (89) |
| LAB 20 | 264 | 245 (93) | 253 (96) | 183 | 171 (93) | 179 (98) |
| LAB 30 | 226 | 225 (100) | 225 (100) | 192 | 189 (98) | 189 (98) |
| LAB 40 | 57 | 57 (100) | 57 (100) | 50 | 39 (78) | 39 (78) |
| LAB 70 | 207 | 196 (95) | 199 (96) | 180 | 168 (93) | 174 (97) |
| LAB 80 | 586 | 281 (48) | 282 (48) | 286 | 276 (97) | 285 (100) |

† See text for explanation of definitions.

**Table 11** Comparison of coliform organism-positive* sample rates by MLSB and Colilert QuantiTray™ (CQT)

| Lab. code | Number of samples | Number samples negative | Positive by both methods | Positive by MLSB only | Positive by CQT only | McNemar's Test‡ $\chi^2$ | McNemar's Test‡ P level† |
|---|---|---|---|---|---|---|---|
| CLAB 1 | 725 | 675 | 50# | 0 | 0 | - | - |
| CLAB 2 | 217 | 202 | 4 | 0 | 11 | 10.0 | < 0.01 |
| CLAB 3 | 2000 | 1970 | 23 | 5 | 4 | 0.03 | > 0.05 |
| CLAB 4 | 885 | 854 | 24 | 2 | 5 | 0.90 | > 0.05 |
| CLAB 5 | 521 | 510 | 6 | 1 | 4 | 1.25 | > 0.05 |
| CLAB 6 | 1660 | 1608 | 46 | 1 | 5 | 2.45 | > 0.05 |
| CLAB 7 | 1476 | 1458 | 14 | 3 | 1 | 0.56 | > 0.05 |
| CLAB 8 | 412 | 406 | 4 | 0 | 2 | 1.13 | > 0.05 |
| CLAB 9 | 675 | 665 | 7 | 0 | 3 | 2.08 | > 0.05 |
| CLAB 10 | 1995 | 1858 | 100 | 26 | 11 | 5.68 | < 0.05 |

* Presumptive coliforms recorded by at least one method.
‡ McNemar's test for the significance of change.
† Significance at p = 0.05, 0.01 or 0.001.
# Results from *E. coli*-positive AQC samples. These were not included in the statistical treatment.



**Table 12** Comparison of E. coli-positive* sample rates by MLSB and Colilert QuantiTray™ (CQT)

| Lab. code | Number of samples | Number samples negative | Positive by both methods | Positive by MLSB only | Positive by CQT only | McNemar's Test‡ $\chi^2$ | McNemar's Test‡ P level† |
|---|---|---|---|---|---|---|---|
| CLAB 1 | 725 | 675 | 50# | 0 | 0 | - | - |
| CLAB 2 | 217 | 210 | 2 | 0 | 5 | 4.05 | < 0.05 |
| CLAB 3 | 2000 | 1993 | 3 | 2 | 2 | 0.00 | > 0.05 |
| CLAB 4 | 885 | 863 | 13 | 6 | 3 | 0.70 | > 0.05 |
| CLAB 5 | 521 | 519 | 2 | 0 | 0 | - | - |
| CLAB 6 | 1660 | 1617 | 42 | 1 | 0 | 0.25 | > 0.05 |
| CLAB 7 | 1476 | 1467 | 7 | 2 | 0 | 1.13 | > 0.05 |
| CLAB 8 | 412 | 409 | 2 | 0 | 1 | 0.25 | > 0.05 |
| CLAB 9 | 675 | 671 | 2 | 1 | 1 | - | - |
| CLAB 10 | 1995 | 1893 | 79 | 21 | 2 | 14.90 | < 0.001 |

* Presumptive *E. coli* recorded by at least one method.
‡ McNemar's test for the significance of change.
† Significance at p = 0.05, 0.01 or 0.001.
# Results from *E. coli*-positive AQC samples. These were not included in the statistical treatment.

**Table 13** Numbers of coliform organisms and E. coli isolated by MLSB and Colilert QuantiTray™ *

| Lab. code | Number of coliform organisms isolated by MLSB at 37°C | Number of coliform organisms isolated by QuantiTray™ | Number of *E. coli* isolated by MLSB at 44°C | Number of *E. coli* isolated by QuantiTray™ |
|---|---|---|---|---|
| CLAB 1† | 2701 | 3089 | 2615 | 3089 |
| CLAB 2 | 16 | 65 | 0 | 18 |
| CLAB 3 | 160 | 218 | 8 | 7 |
| CLAB 4 | 222 | 411 | 227 | 199 |
| CLAB 5 | 39 | 43 | 17 | 5 |
| CLAB 6 | 870 | 2096 | 935 | 1677 |
| CLAB 7 | 156 | 340 | 29 | 18 |
| CLAB 8 | 22 | 34 | 11 | 8 |
| CLAB 9 | 33 | 121 | 5 | 5 |
| CLAB 10 | 1910 | 2654 | 1504 | 1162 |

* Confirmed counts for MLSB, ß-galactosidase and ß-glucuronidase positive wells for Colilert QuantiTray™.
Excludes greater than results by either or both methods.
† Data from 50 *E. coli*-positive AQC samples (all other samples negative).

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# Evaluation trials for two media for the simultaneous detection and enumeration of *Escherichia coli* and coliform organisms

*Methods for the Examination of Waters and Associated Materials*