An evaluation of presence-absence tests for coliform organisms and Escherichia coli 1996

Methods for the Examination of Waters and Associated Materials

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Contents

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 more 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 methods is often shown at the front of publications 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.

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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 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 members associated with methods are listed at the back of booklets.

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

Dr D WESTWOOD
Secretary

27 December 1995

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'.

Glossary

IPTG	1-isopropyl-β-D-1-thiogalactopyranoside
LPW	lactose peptone water
MB	MacConkey broth
MLSB	membrane lauryl sulphate broth
MMGM	minerals modified glutamate medium
MF	membrane filtration
MPN	most probable number
MUG	4-methylumbelliferyl-β-D-glucuronide
ONPG	ortho-nitrophenyl-β-D-galactopyranoside
PA	presence-absence
PWS	private water supplies
SRW	surface (recreational) water
SUW	surface water
SW	spring water
TW	tryptone water
UK	United Kingdom
UV	ultraviolet
X-GAL	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Summary

Presence-absence (PA) tests, the most probable number (MPN) test using minerals modified glutamate medium (MMGM) and the membrane filtration (MF) technique using membrane lauryl sulphate broth (MLSB) were compared for their efficiencies in detecting coliform organisms in 100 ml volumes of water. An efficient test for drinking water needs to be sensitive and specific for the target organism, in this case the coliform organism, including *Escherichia coli* (*E. coli*). For regulatory purposes, the detection limit must be as close as possible to 1 target organism per 100 ml of water and false positive and negative reactions should be minimised. New tests should therefore have performance characteristics comparable to those of the MF and MPN methods.

Following a comprehensive review of published reports, ie phase one of the study, four PA tests were selected for evaluation. These were

(i) lactose-lauryl tryptose-tryptone broth (APHA PAB, Bacto Presence Absence Broth, Difco);
(ii) Fluorocult LMX broth (Merck);
(iii) Colitrace (Bradsure Biologicals); and
(iv) Colilert (Palintest Ltd).

The mechanisms of detection of coliforms and *E. coli* by the tests were also reviewed in relation to their likely ability to detect coliform organisms as defined in Report 71 (1983) ie "old definition" producing acid and gas from lactose, and coliform organisms as defined by the revised Report 71 (1994) ie "new definition" producing ß-galactosidase.

In phase two of the study, three batches of 200 duplicate simulated water samples were prepared containing respectively a strain of *E. coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. For maximum statistical power, the batches were prepared so that about 50% of samples were expected to be positive and contain 1 or more organisms per 100 ml. A total of 100 samples from each batch were tested in each of two laboratories. All of the tests examined performed satisfactorily, although for the strain of *E. coli*, three of the test kits (PAB, LMX and Colilert) gave a lower proportion of positives than MF. For two of the test kits (LMX and Colilert) this was statistically significant, see Table 2.

The four PA tests, and MMGM used in a PA format rather than the MPN format, were then compared with MF in a field trial at eight laboratories, ie phase three of the study. The water used was obtained from natural sources, mostly private water supplies (PWS) expected to yield water containing low numbers of coliform organisms similar to those created in the simulated samples such that about 50% of samples would be expected to be positive. A total of 1409 samples were examined using each method. It was possible to compare the performances of detecting coliform organisms and *E. coli* since some of the coliform organisms present were *E. coli*.

Contrasting findings from different geographical areas mean that an aggregated summary should be interpreted with the qualifications expressed in this booklet. Overall, Colilert and LMX detected more coliform organisms than MF when incubated for the maximum recommended times of 28 and 48 hours respectively. MMGM, used as a PA test, and Colitrace gave comparable results to MF at 48 hours but PAB was less effective, see Table 4. Thus, if one is only interested in the final result MMGM, Colitrace, Colilert and LMX all gave a comparable number of positives to MF. However, MF gave a faster presumptive positive result for coliform organisms at the low levels of contamination studied here.

For *E. coli*, Colilert reported fewer positives than LMX and Colitrace, see Table 11. There were also water sources where Colilert and, to a lesser extent, Colitrace failed to detect *E. coli* which were detected by MF and LMX. The results indicate that there is no PA test kit that is best suited for all locations for both coliform organisms and *E. coli*. As there can be marked ecological differences between sources it will be important that any intended use of a PA test is validated in each geographical area.

An evaluation of presence-absence tests for coliform organisms and *Escherichia coli*

1 Literature review and preliminary assessment of available presence-absence tests

1.1 Introduction

The presence of coliform bacteria, in particular *E. coli*, is the principal microbiological parameter used to determine the sanitary quality of drinking water. Traditionally in Britain these organisms have been measured quantitatively by means of the multiple tube MPN technique or by MF. The legislative requirement in the European Community is for there to be no coliform organisms or faecal coliforms in 100 ml of drinking water (EEC Directive 80/778). These standards have been incorporated into United Kingdom (UK) legislation in the Water Supply (Water Quality) Regulations 1989. In the UK, *E. coli* is considered the only true faecal coliform so that for practical purposes the requirements in the UK is for coliforms and *E. coli* to be absent in 100 ml.

Usually in the UK, when the MPN technique is applied to waters expected to be of potable quality, one 50 ml and five 10 ml volumes of the water under investigation are added to equal volumes of a suitable double strength differential medium. Currently, the medium of choice is MMGM (PHLS 1969) for both coliforms and *E. coli*. After incubation, tubes with reactions typical of coliforms and *E. coli* are subcultured to appropriate media for confirmation of the identity of the organisms. For the purposes of further discussion, the initial phase of the test is termed the presumptive phase. In the MF method, 100 ml of water are filtered through a membrane which is then placed on the surface of a filter pad soaked in an appropriate medium. The medium of choice is MLSB (PHLS/SCA 1980) for both coliforms and *E. coli*. In both methods, presumptive positives have to be confirmed and the whole process takes 2–3 days.

As the requirement is for the absence of coliforms and *E. coli* in 100 ml of water, a simple qualitative test for the presence or absence of the target organisms in a 100 ml volume of the water could satisfy part of the requirements for routine monitoring of waters expected to be of potable quality provided the test has a sensitivity and specificity at least as good as the MPN and MF methods. A simple qualitative test for detecting the presence of coliforms in a volume of water is termed a PA test and its use was first suggested by Weiss and Hunter (1939). There was little attempt to develop PA tests until the work of Clark (1968). In North America, the concern with improving the quality of drinking water supplies, particularly small ones, with the concomitant requirement for increased monitoring, often at remote sites, has led to an increased interest in the use of PA tests for detecting coliform bacteria in drinking water.

The first attempts to evaluate the use of PA tests for routine monitoring utilised modifications of the media used in the presumptive phase of the MPN methods. Firstly, Clark (1968) used a modified MacConkey broth (MB) enriched to improve acid and gas production by coliforms. Due to the variable performance of different batches of bile salts, MB was superseded by a lactose-lauryl tryptose-tryptone broth (Clark *et al*, 1982). This medium has become known as PA broth or APHA PAB, is available commercially, and is listed as the medium of choice by the American Public Health Association (APHA, 1992) for the presumptive phase of the test. Throughout this document it will be referred to as PAB. The Association also lists lauryl tryptose broth as an alternative presumptive phase medium. Further trials, which are discussed below, confirmed that the PA test using PAB may maximise coliform detection in samples containing many organisms that could overgrow coliform colonies and cause

problems in detection using the MF method. The outcome of these trials was that the PA test became accepted as a method for routinely monitoring drinking water supplies in the USA (Environmental Protection Agency, 1989).

The MF, MPN and PA methods described so far all require the use of confirmatory tests after the initial presumptive phase of the test. A complete analysis can require 24 to 72 hours for a final result. These tests are all based on the production of acid from lactose at 37 °C by coliforms, and acid and gas from lactose and indole production at 44 °C by *E. coli*. Edberg *et al* (1988) suggested that chromogenic or fluorogenic substrates specific for particular enzymes could be incorporated in a liquid test medium for water testing. The choice of substrates for enzymes specific for the target organisms could enable the test to become a one step test. The test medium contains the substrates ortho-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) which are specific for β-galactosidase and β-glucuronidase respectively. The production of a yellow colour indicates the presence of β-galactosidase which is present in coliforms, and blue-white fluorescence under ultraviolet (UV) irradiation (366nm) indicates β-glucuronidase activity which is found in *E. coli* but not other common coliforms. The first report of the use of Colilert as a PA test was made by Edberg *et al* (1989) and subsequently there have been many further trials of the method which are described below.

Other tests have also been developed recently for water bacteriology involving the incorporation of defined chromogenic and/or fluorogenic substrates in media, but these are essentially different from Colilert in that substrates are incorporated in relatively nutrient rich media which are usually developments of existing media. Colilert is presently unique in having the substrates in a mineral medium with practically no other added nutrients. Thus the currently available PA tests can be broadly divided into three categories:

(i) conventional lactose based nutrient rich;

(ii) nutrient rich defined substrate tests; and

(iii) nutrient poor defined substrate tests.

1.1.1 Aim

Outside North America there have been few published trials of PA tests. However, the PA test using MMGM is accepted in the UK for testing water leaving a treatment works provided it is not used for this purpose more than twice a week (DOE, 1989). The overall objective of the study reported in this booklet was to evaluate currently available PA tests for coliform organisms for their detection efficiency and to compare their performance with that of traditional biochemical methods. The first part of this study was to:

(i) identify suitable and promising PA methods and their scientific bases; and

(ii) assess the results obtained in published work against old and new definitions of coliforms and *E. coli* used in the UK, and determine any possible differences in results that might result.

1.2 Definitions

1.2.1 Coliforms

The Bacteriological Examination of Drinking Water Supplies 1982 (Report 71, 1983) defines coliform organisms as:

> "Gram-negative, non-sporing rod-shaped bacteria, capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface active agents with similar growth-inhibiting properties, which are able to ferment lactose with the production of acid and gas within 48 hours at 37 °C. They are also oxidase-negative"

Coliform organisms which have the same fermentative properties at 44 °C are described as "thermotolerant".

This definition is referred to as the "old definition".

Guidance on Safeguarding the Quality of Public Water Supplies (DOE, 1989) defines coliform organisms as:

> "members of a genus or species within the family Enterobacteriaceae, capable of growth at 37 °C, and normally possessing β-galactosidase".

This is the definition of coliform organisms that has been incorporated into the revised Report 71, *The Microbiology of Water 1994—Part 1—Drinking Water*, and is referred to as the "new definition".

1.2.2 *Escherichia coli*

In the 1983 Report 71, *E. coli* was defined as:

> "a coliform organism which ferments lactose or mannitol at 44 °C with the production of acid and gas within 24 hours and which produces indole from tryptophan".

A small proportion of strains of *E. coli* do not fall within this definition because they do not express these characteristics at 44 °C or they are anaerogenic, ie do not produce gas. They will, however conform with the new definition of coliform organisms. When identified by reliable methods such as commercial kits or well established traditional microbiological techniques these micro-organisms are recognised as *E. coli* and have the same sanitary and operational significance.

1.2.3 Comparison of classification of members of Enterobacteriaceae using the old and new definitions of coliforms

In order to contrast the likely classifications, the biochemical characteristics of the members of the Enterobacteriaceae were compared using the data matrices of Farmer *et al* (1985, 1991) and Brenner (1984). These data matrices are largely based on the characteristics of isolates from clinical material. By comparison there is relatively little published information on the characteristics of environmental isolates and no data matrices have been produced. As anticipated, an appraisal of the data matrices reveals that a higher proportion of the Enterobacteriaceae would be classified as a coliform according to the new definition. This is summarised in Table 1. Whether these potential discrepancies would result in an apparently higher incidence of coliform failures in drinking waters depends on the relative frequency of the relevant strains in water and on the expression of the characteristics in the isolation media used. The majority of the coliform organisms isolated from water, apart from *E. coli* are usually species of *Enterobacter, Klebsiella, Citrobacter* and *Serratia* (Clark and Pagel, 1977) and, in general, the discrepancies within these groups will only be found in a very small proportion of the commonly isolated types. As detailed below, published evidence of data suggests that with Colilert the utilisation of β-galactosidase as a marker rather than lactose fermentation does not appear to increase significantly the detection of coliforms. It cannot, however, be assumed that this would be the case for other PA media. Considering its sanitary significance the classification of *Shigella sonnei* as a coliform in the new definition is a potential advantage but since it would normally be vastly outnumbered by *E. coli* and other coliforms it is unlikely to have any impact, except in exceptional circumstances. The same argument would also apply to *Yersinia enterocolitica* which is probably more likely to be overgrown by other organisms.

1.3 PA Test Kits

1.3.1 Modified MacConkey Broth

MacConkey broth modified by the addition of 10 g of tryptone per litre was the medium first used by Clark (1968) in studies of the use of PA tests. Its performance was compared with MF using m-Endo MF broth. The two methods were compared using 8764 drinking water samples mostly collected in southern Ontario from a wide variety of sources treated by a variety of purification methods. Statistical evaluation showed the PA test was more sensitive for detecting lower levels of contamination

than the MF technique. Many of the confirmed PA positive results came from PA bottles that had extended incubation of 2 to 5 days. Later, it was reported (1980) that this PA method was more effective than MF in detecting indicator organisms in the presence of high background counts. The modified MB is nutrient rich containing peptone (20 g/l), tryptone (5 g/l) and lactose (20 g/l) and coliforms are detected by the formation of acid and gas from lactose.

1.3.2 Lactose-lauryl tryptose-tryptone broth (APHA PAB, Bacto presence-absence broth)

Clark *et al* (1982) developed this medium as an alternative to the modified MB because the components were more readily available from suppliers. The formulation was made up by combining the components of lactose broth, lauryl tryptose broth and tryptone and is now available commercially. Clarke *et al* (1982) used this formulation from 1979 in their studies of the characterization of indicator bacteria in water. The medium is nutrient rich and coliforms are detected by the production of acid and gas from lactose and thus will detect organisms conforming to the 'old' definition of coliforms. Confirmation of coliforms and *E. coli* requires subculture to appropriate confirmatory tests.

Jacobs *et al* (1986) found PAB more sensitive than MF using M-Endo broth, and slightly more sensitive than the MPN method using lauryl tryptose broth for detecting coliforms in 1483 different drinking water samples from small community water systems. The PAB displayed high confirmation efficiency when both acid and gas production were present, but when only acid was present, coliforms were not usually found to be present.

Rice *et al* (1987) compared lauryl tryptose broth, lactose broth, PAB, lactose broth with twice the amount of lactose, and lauryl tryptose broth with twice the amount of sodium lauryl sulphate as MPN media in comparison with MF using M-Endo LES agar and mT7 medium. It was reported that there was no significant difference between the liquid media in the MPN method but the broth media gave significantly higher counts than mT7.

Bancroft *et al* (1989) compared PAB with MF using m-Endo LES broth for coliform detection in small non-chlorinated water distribution systems. Samples were collected from 40 locations at monthly intervals in one distribution system. Although more samples were positive by the PA method, the difference was not significant. Pipes *et al* (1986) also compared the PA test and MF by examining 2601 samples from small water supplies. They found 569 positive samples of which 23.2% were positive by the PA test alone and 26.7% by the MF test alone. This difference was not statistically significant.

Martins *et al* (1991) compared PA tests using modified MB and PAB with the MF and MPN methods using fifty samples of sewage polluted river water and artificially contaminated spring water. It was reported that there was no significant difference between the tests for the detection of coliforms but the modified MB was less efficient than the PAB for the detection of faecal coliforms.

Rice *et al* (1989) combined the data from four studies. These included the work of Pipes *et al* (1986) and Jacobs *et al* (1986) as discussed above, together with two other studies. The results were compared based on the number of positive samples detected by each method. The combined recoveries showed the PA test using PAB detected significantly higher numbers of samples with coliforms than either the MPN or MF methods. They concluded that the PA test offers a viable alternative for compliance monitoring using the frequency-of-occurrence approach, ie compliance based on the fraction of samples containing coliforms during a given period.

1.3.3 Defined Substrate Media with Low Nutrients

1.3.3.1 Colilert PA

Edberg *et al* (1988) developed Colilert with the following aims: to enumerate specifically 1 coliform/100 ml in a maximum of 24 hours; to enumerate simultaneously and specifically 1 *E. coli* per 100 ml in the same test; to be unaffected by other heterotrophic organisms found in the sample; not to require confirmatory tests; to grow injured coliforms; to be easy to inoculate, and to be very easy to interpret. In the PA presentation, the components of the test medium are packaged in individual sachets sufficient for a 100 ml sample of water. The contents of the sachet are added to the water sample in a non-fluorescent glass or plastic container and incubated at 37 °C for 24 hours. The medium contains a variety of salts, amphotericin B (1 mg/l), ONPG (500 mg/l), MUG (75 gm/l) and solanium (50 mg/l). The latter is a plant extract that is said to act as an emulsifier. It is clear that essentially the only nutrients are ONPG and MUG. Coliforms are detected by the presence of a yellow colour in the medium resulting from the activity of β-galactosidase on ONPG. If the container is then exposed to UV irradiation, blue-white fluorescence indicates the breakdown of MUG due to β-glucuronidase which is characteristic of *E. coli*. Thus coliforms are detected on the criterion of β-galactosidase and thus confirm to the new definition of coliforms. The method was developed in the USA and consequently the majority of published trials of this method have been in North America where the standard methods for MPN and MF are not identical to those in the UK.

The first trial of Colilert in PA format was that of Edberg *et al* (1989). This was a national trial of 702 water samples which were examined by Colilert and one other method. Overall there was no significant difference in the result of the Colilert and other tests (94% comparability). There were 358 samples negative in both tests, 302 positive in both, 20 positive by a standard method but not Colilert, and 22 positive by Colilert and not the standard method. The standard method varied from laboratory to laboratory being either MF, MPN or PAB. Where it was recorded, most (31) of the positive samples had coliform counts less than 10 and for 26 it was less than 5. Only one sample contained *E. coli* and this was positive by both methods and contained 1 *E. coli*/100 ml. The remaining coliforms were mostly *Klebsiella, Enterobacter* and *Serratia* species.

Lewis and Mak (1989) compared Colilert with the MF method using mEndo LES agar. It was reported that for 950 samples of treated drinking water the same result was given 97% of the time on the basis of confirmed coliforms. However, only 16 samples were positive and for these, 5 were in agreement, 2 were positive for Colilert but not MF and 9 were positive for MF and not Colilert. Although the sample was very small they also stated "it was disturbing that neither of the positive *E. coli* tests exhibited fluorescence and that *E. coli* could not be isolated from either of the fluorescent Colilert tests." They implemented the use of Colilert for coliform determination but not *E. coli* on the basis of these results.

In a trial using source water which was diluted to give 1 to 20 coliforms/100 ml, Edberg *et al* (1990) compared Colilert in MPN format with the APHA standard MPN technique using lactose tryptose broth. A total of 47 samples were analyzed and Colilert was found to be as sensitive as the standard method. Again there was some indication that Colilert was not as good as the standard method at detecting *E. coli* since only 1% of the isolates detected by Colilert were *E. coli*, whereas the figure was 6% for the standard method. Katamay (1990) compared MF and Colilert in MPN format for 176 water samples including source, effluent and distribution samples. Again the two methods gave similar results and in this trial Colilert detected more *E. coli* (55) than MF (46). Beebe *et al* (1990) compared Colilert in PA format with MF, presumably using mEndo LES agar but this is not clear, using 271 samples of well water. Both methods detected 44 positive results for total coliforms but *E. coli* was detected in 9 samples by MF but only in 6 by Colilert. In addition, they were also unable to isolate *E. coli* from four MUG positive samples as was found by Covert *et al* (1989) and isolated *E. coli* from two samples that were MUG negative. It was reported that Colilert was suitable for monitoring for total coliforms.

Olson *et al* (1991) compared MF using m-Endo LES agar with Colilert and Coliquick in PA formats using 750 water samples from a variety of sources. Coliquick was a defined substrate test similar to Colilert. Olson reported that MF gave more positives (160) than Colilert (127) which was significantly different and that high background counts of other bacteria did not interfere with the results. Clark *et al* (1991) compared Coliquick and Colilert with MF using MFC broth for the detection of *E. coli* in 83 treated water samples from an open reservoir and 32 untreated surface water samples. For the treated water samples Colilert and Coliquick were significantly less sensitive than MF which detected 43 positives in contrast to 18 by Coliquick and 11 by Colilert. As with the study of Olson *et al* (1991) the probability of a false negative result with Colilert was highest with low numbers of *E. coli*.

Clark and El-Shaarawi (1993) compared PAB, MF using both m-Endo LES (for coliforms) and M-TEC (for *E. coli*) agars with Colilert, Coliquick, and another PA medium based on PAB but supplemented with MUG. A river water was used, diluted in a buffer which was not specified. With the exception of Coliquick, all the PA methods gave similar results overall for the recovery of coliforms. In one laboratory, Colilert gave a significantly lower recovery of faecal coliforms but this was not apparent in the other laboratories.

All of the studies detailed so far were performed in North America and compared Colilert and other PA methods with methods used in the USA and Canada which are not the same as those used in the UK where MMGM is used for the MPN method and MLSB for MF. Schets *et al* (1993) compared Colilert with the MPN method using MMGM and MF using tryptone bile agar for detecting *E. coli*. It was reported that the average recovery of *E. coli* with Colilert was 26% and that for coliforms was 35% when compared with the Dutch methods. In samples with low numbers of target organisms, Colilert gave false negative results and was considered an unsuitable alternative to the standard methods.

In an unpublished trial in England and Scotland carried out by members of, and under the auspices of the Standing Committee of Analysts, four laboratories examined a total of 156 samples by MF using MLSB compared with Colilert. The Colilert test gave poor performance in detecting coliforms from treated waters and there was also a suggestion that *E. coli* were found in lower numbers, see Appendix C.

It is apparent from the above discussion that Colilert is probably comparable with the US standard methods for detecting coliforms in water, but there is some evidence that this is not true for *E. coli*, which is also a coliform and represents an appreciable proportion (greater than 10%) of coliforms detected in water in most studies. The data from the Netherlands suggests that Colilert will perform in a similar way in comparison with British and European methods.

1.3.4 Defined Substrate Media with High Nutrient

1.3.4.1 Colitrace

Colitrace is MMGM supplemented with MUG. It thus has the relatively high concentration of nutrients, albeit defined, found in MMGM. Presumptive coliforms are detected by the production of acid and gas from lactose and therefore conforms to the old definition of coliforms. In contrast, *E. coli* is detected by the presence of β-glucuronidase. There are no published reports of the use of this medium but one might expect it to behave like MMGM in terms of the ability to detect coliforms.

1.3.4.2 Fluorocult LMX broth and others

A number of other media have been produced that are essentially traditional media modified by the addition of ONPG, MUG or other chromogenic or fluorogenic substrates. A range of these is commercially available. These include: Fluorocult BRILA broth (BRILA-MUG, Brilliant green Lactose Bile Broth + MUG) which has been used for bathing waters but not drinking waters; Fluorocult DEV lactose peptone broth (DEVMUG) which is the standard medium used in Germany for the detection of *E. coli* and coliforms in drinking water but supplemented with MUG; Fluorocult

lauryl sulphate broth (LSBMUG, lauryl sulphate broth + MUG), and Fluorocult LMX broth. The publications dealing with these media involve testing with bathing waters and not drinking waters.

Muller *et al* (1992) testing bathing waters found the brilliant green in BRILA-MUG too inhibitory and noted that without this component the medium was essentially MB + MUG. For *E. coli* exposed to sea water for 7 days, DEV-lactose-peptone broth gave slightly better recovery than BRILA-MUG and was slightly better than LSBMUG (Kolbeck *et al* 1992). After only 24 hours exposure, DEV-lactose-peptone broth was slightly worse than the other media.

LMX broth was developed by Manafi and Kneifel (1989) to simultaneously detect coliforms and *E. coli*. It has subsequently been modified and is now marketed as LMX Broth modified according to Manafi and Ossmer (*sic*). This medium contains (g/l) tryptose 5.0; sodium chloride 5.0; sorbitol 1.0; tryptophan 1.0; dipotassium hydrogen phosphate 2.7; potassium dihydrogen phosphate 2.0; sodium lauryl sulphate 0.1; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) 0.08; MUG 0.05; 1-isopropyl-β-D-1-thiogalactopyranoside 0.1 (IPTG). It can be seen that the medium contains a moderate amount of nutrient together with MUG, X-GAL and the β-galactosidase inducer IPTG which should have an amplification effect on the synthesis of the enzyme. The chromogenic substrate X-GAL produces a green colour when cleaved by β-galactosidase and serves the same role as ONPG. Thus in a single tube one can determine the presence of β-galactosidase, β-glucuronidase and the production of indole by overlaying the medium with Kovacs reagent. The medium will detect coliforms according to the new definition and is unique among the media discussed here in containing a β-galactosidase inducer. Although Manafi and Kneifel (1989) mention the use of the original LMX broth for testing 104 waters there are no clear details given of how the method compared with other methods.

1.4 Choice of Trial Media

APHA PAB and MMGM are the media recommended for PA tests in North America and the UK respectively, but have never been compared. Consequently APHA (PAB) broth was included as one of the test media. Since PAB broth was selected instead of MacConkey broth following extensive trials in North America (see above) it was not considered advantageous to include MacConkey broth in this trial.

Lauryl tryptose broth has been shown to be less effective than MMGM (PHLS/SCA, 1980) at least for chlorinated waters and especially if numbers of coliforms or *E. coli* are low. Therefore lauryl tryptose broth and variants of it containing MUG such as LSBMUG were excluded from this trial.

The effectiveness of BRILA-MUG relative to LSBMUG varies from study to study. Kolbeck *et al* (1992) found BRILA-MUG superior to LSBMUG whereas Schindler (1991) concluded LSBMUG was superior. As already noted, BRILA-MUG broth has only been used for bathing waters, is quite inhibitory and without brilliant green is comparable to MB which has already been discounted. For these reasons, it was concluded that it was unlikely to be worthwhile including BRILA-MUG in the trial.

LMX broth is fundamentally different from the other nutrient rich media in having IPTG as an inducer of β-galactosidase rather than lactose itself. This medium was included in the trial because it has not been subjected to any extensive trials in drinking waters and is quite different from lauryl tryptose broth from which it was developed.

Although Colitrace is simply MMGM supplemented with MUG, and MMGM was included as a reference medium in MPN format, Colitrace was included in the trial because the inclusion of MUG may affect the performance of the medium. There was also interest to see how this medium compares to Colilert and LMX broth at detecting low numbers of *E. coli* which appears to be a potential problem.

There is an absence of published trials comparing Colilert with UK methods which are different and possibly less inhibitory than those used in North America. Colilert is the only low nutrient defined substrate test currently available. It has been extensively tested in North America and found to be equivalent, in general, with their standard methods for the detection of coliforms although possibly not for *E. coli* or at low concentrations of coliforms. Following its acceptance in the USA it was included in the trial.

To summarise, the methods included in this study were:

1. MF using MLSB (Unipath) as in Report 71
2. The MPN method using MMGM (Unipath) as in Report 71
3. APHA PAB (Difco)
4. Fluorocult LMX broth (Merck)
5. Colitrace (Bradsure Biologicals)
6. Colilert (Idexx)

2 Evaluation of presence-absence tests using simulated samples

2.1 Introduction

In this phase of the evaluation, three batches of 200 simulated samples were prepared and examined. The aim was to assess quickly the tests against three separate and different coliforms in laboratory controlled situations.

2.2 Methods

The following organisms were used:

Escherichia coli (*E. coli*)
Enterobacter cloacae (*Ent. cloacae*)
Klebsiella pneumoniae (*K. pneumoniae*)(a thermotolerant strain)

Each organism was prepared as a separate batch of 200 replicate samples. Preliminary work was carried out in order to ascertain the level of inoculation required in order to achieve a level whereby 50% of the samples showed a positive response and 50 % a negative response. Once this had been ascertained, the required organism was inoculated into single strength nutrient broth and incubated at 37 °C. After several hours, an aliquot was transferred to 200 ml of preservation medium (nutrient broth containing 1.8% boric acid). This was well mixed on a magnetic stirring device, and a Miles and Misra surface colony count performed. The suspension was refrigerated overnight.

On the following day, the suspension was again well mixed and an aliquot transferred to a larger volume of preservation medium, sufficient to enable distribution of 200 samples.

The volume transferred was such that when 3 ml of the distributed sample was added to 1000 ml of sterile distilled water, there would be between 1 and 2 organisms per 100 ml of water.

To check that the number of organisms present was at a suitable level for distribution, 3 ml volumes of the well mixed suspensions were added to 24 one litre volumes of sterile distilled water. Duplicate 100 ml volumes from each one litre water sample were examined by MF as recommended in Report 71 (1983). The suspension was refrigerated overnight.

On the following day, if the percentages of positive and negative samples were satisfactory, the well mixed suspensions were distributed into 200 sterile 8 ml "bijoux" bottles full to the brim. If the percentages were deemed unsatisfactory, then a new volume of preservation medium was inoculated with a slightly modified volume of the well mixed original refrigerated 200 ml suspension, and the filtration process repeated. This new suspension was refrigerated overnight and distributed the following day.

The samples for examination by laboratory 4 were refrigerated immediately after distribution. The samples for examination by laboratory 5 were despatched by courier and refrigerated within several hours of distribution.

2.3 Statistical Methods

The statistical methods are described in detail in sections 3.4 and 3.5. Comparisons were made of the PA result from the 6 methods using the recommended manufacturers' timing and indication:

(i)	MF	—	18 hour result
(ii)	MPN	—	48 hour result
(iii)	PAB	—	48 hour acid result
(iv)	LMX	—	48 hour green result
(v)	Colitrace	—	48 hour yellow result
(vi)	Colilert	—	28 hour yellow result

Methods (i) and (ii) were regarded as the reference methods and methods (iii) to (vi) were compared with them for each of the three batches of trial organisms.

2.4 Results

The results are summarised in Table 2. From 200 *E. coli* samples, the proportion showing yellow colonies by MF was 0.70 (ie 70%). The proportion showing growth in the MPN tube (MMGM) method was 0.715 (ie 71.5%). This excess of 0.015 over MF was not statistically significant and was probably due to chance (ie sampling error). Table 2 shows that three of the four kits gave a lower proportion of positives than MF and for two of them (LMX and Colilert) this was statistically significant.

From 200 *Ent. cloacae* samples, the proportion showing yellow colonies on MF was 0.725 (ie 72.5%). All the other methods found slightly higher proportions of samples positive, but these differences were not statistically significant.

From 200 *K. pneumoniae* samples, the proportion showing yellow colonies on membrane was 0.54 (ie 54%). For Colitrace, the proportion was 0.66 and this was the only method which gave significantly different results from MF for this organism.

Detailed results from each laboratory appear in Appendix A. In Appendix A, Table A1 shows that the MF and MPN results were comparable for all three samples and there were no systematic differences.

Tables A2–A5 show paired results for each of the 4 trial methods (iii–vi) compared with MF and MPN, by laboratory. If any of the trial methods had performed consistently badly in both laboratories, then it would have been justified to drop that test method from phase 3 of the study. This was not the case, although 3 of the trial methods showed poor recovery rates for *E. coli*. Only Colitrace performed as well as MF and MPN for *E. coli*. All methods performed adequately with *Ent. cloacae* and *K. pneumoniae*. With *K. pneumoniae*, Colitrace achieved a significantly higher proportion of positives than MF, though not significantly higher than MPN.

3 Field trial

3.1 Principle

Eight Public Health Laboratories participated in the trial. Water samples were collected from natural sources that, from previous results, were expected to give counts of 0 to 5 coliforms per 100 ml. Each sample was examined for the presence of coliforms by MF using MLSB, and the PA techniques using MMGM, PAB, LMX, Colitrace and Colilert.

3.2 Materials

Membrane Lauryl Sulphate Broth (MLSB) Oxoid MM615 Batch 063 52108 (expiry January 1998) or batch 063 52702 (expiry May 1998). A total of 76.2 g were suspended in 1 litre of distilled water, dispensed in volumes of 50 ml to 250 ml and sterilized by autoclaving at 121 °C for 15 minutes.

Minerals Modified Glutamate Medium (MMGM) Minerals Modified Medium Base Oxoid CM607 batch 057 52184 (expiry January 1996) plus sodium glutamate L124 batch 057 67323 (expiry November 1997). Triple strength medium was prepared by adding 34.0 g base CM607 and 19.0 g sodium glutamate L124 to 1 litre of distilled water containing 7.5 g ammonium chloride. The pH was adjusted to 6.7. The medium was then dispensed in 50 ml amounts in bottles of at least 150 ml capacity and sterilized by autoclaving for 10 minutes at 116 °C. The pH was checked after cooling to confirm it had remained at pH 6.7 $\pm$ 0.1.

Presence-Absence Broth (PAB) Difco product code 0019-17-0, batch 27478 (expiry January 1996) or batch 26494JA (expiry June 1997). 91.5 g were dissolved in 1 litre deionised water and dissolved by warming gently. 50 ml amounts of medium were dispensed into screw capped bottles of at least 150 ml capacity and sterilised by autoclaving at 121 °C for 12 minutes.

Fluorocult LMX Broth (LMX) Merck batch number 240 V 260120 (expiry October 1997). Triple strength broth was prepared by dissolving 51 g in 1 litre demineralised water, adjusting the pH to 6.8 $\pm$ 0.1 at 25 °C, dispensing in 50 ml amounts in non-fluorescent screw-cap containers of at least 150 ml capacity and sterilizing at 121 °C for 15 minutes.

Colilert Palintest batch 7052 (expiry June 1994). Packed in sachets. Each sachet containing sufficient medium for adding to a 100 ml water sample in a screw capped non-fluorescent container of at least 150 ml capacity.

Colitrace Bradsure Biologicals batch 150493 (expiry September 1994). Packed as ready to use medium in 100 ml amounts in sterile plastic wide mouthed containers.

Media for confirmation tests Nutrient agar, MacConkey agar, lactose peptone water containing Durham tubes, tryptone water, Kovacs indole reagent and oxidase reagent were all prepared according to Report 71 (1983)

Identification kits Where applicable, strains were identified using API20E or API20NE (bioMérieux, Marcy l'Etoile, France).

Membranes and pads Millipore cellulose ester membranes pore size 0.45 μm, lot number H2MM97377 and pads, lot number H2MM97271.

Sample bottles Sterile 1 litre glass or plastic bottles containing 1 ml of 1.8% (m/v) sodium thiosulphate ($Na_2S_2O_3.5H_2O$).

3.3 Method

One litre samples of water were collected from sources expected to contain low numbers of coliforms.

3.3.1 Inoculation of tests

With large numbers of tests to be inoculated it was important to ensure there was no time bias in the results. Consequently, for each sample, all the methods were inoculated as close together in time as possible and the order of the inoculation of the tests was randomised so that one method was not always the first inoculated.

Each sample was mixed well by shaking and inverting at least 20 times and the following tests conducted.

Reference method:—

Membrane Filtration—A 100 ml volume of sample was examined for coliforms by MF following the methods in Report 71, section 7.9 (1983) using MLSB. Membranes were incubated at 30 °C for 4 hours followed by 15 $\pm$ 1 hours at 37 $\pm$ 0.5 °C.

Test PA Methods:—
Each PA method requires a bottle to be filled with a measured volume of 100 ml of water sample. This was achieved by use of a sterile measuring cylinder or by weighing 100 g quantities with a taring weighing machine.

MMGM, PAB and LMX—A 100 ml volume of sample was added to 50 ml of triple strength medium, the container sealed and inverted two or three times to mix the contents before incubating at 35–37 °C and examining after 24 and 48 hours.

Colitrace—A 100 ml volume of sample was added to the Colitrace container, the container sealed and the contents mixed by inverting two or three times before incubating at 35–37 °C and examining after 24 and 48 hours.

Colilert—A 100 ml volume of sample was added to a sterile non-fluorescent container. The contents of one Colilert sachet were added aseptically taking care not to touch the opening of the pack. The container was sealed and the contents mixed vigorously to dissolve the reagent. Incubation at 35–37 °C was commenced within 30 minutes. The tubes were examined after 24 hours and, in the case of weak or doubtful reactions, the tubes were incubated a further 4 hours and read again.

The formulations represent those in use at the time of the study, ie October 1993–March 1994.

3.3.2 Recording results

Membrane filtration—All presumptive coliform (yellow, lactose-fermenting) colonies were counted. Each presumptive colony was sub-cultured to two tubes of lactose peptone water (LPW) containing Durham tubes to detect gas production; a tube of tryptone water (TW); a nutrient agar plate and a MacConkey agar plate. The TW and one LPW were incubated at 44 ± 0.5 °C for 24 hours and the remaining media at 37 °C for 24 hours. The MacConkey and nutrient agar plates were examined for purity and the oxidase test performed from the nutrient agar plate. Acid and gas production in the LPW, and indole production in TW were recorded and the strains were identified as described in the tabulation:

Lactose 37 °C		**Lactose** 44°C		**Indole** 44°C	**Oxidase**	**Identification**
acid	gas	acid	gas			
+	+	–	–	NA	–	Coliform by old and new definition
+	–	–	–	NA	–	Coliform by new definition only
+	+	+	+	+	–	*E. coli*

(NA not applicable)

MMGM The presence or absence of acid and gas production at 24 and 48 hours was noted.

PAB The presence or absence of acid and gas production was recorded after 24 and 48 hours incubation. A distinct yellow colour indicates acid production. Gas production can be observed by a foaming reaction when the bottle is shaken gently.

LMX The production of β-galactosidase (green or blue-green colour) and β-glucuronidase (fluorescence under long wave UV irradiation) was noted after 24 and 48 hours.

Colitrace Acid production and fluorescence was recorded after 24 and 48 hours.

Colilert Observations were noted after 24 hours. A yellow colour indicates the activity of β-galactosidase. The colour should be uniform throughout the sample—if not, it was inverted to mix before reading. Under UV irradiation, fluorescence indicates the activity of β-glucuronidase. The manufacturers state that occasional doubtful samples may be incubated for an additional 4 hours, so this was carried out routinely.

3.3.3 Identifications of isolates from PA tests

When no coliforms, as defined by the old and new definitions, were detected by MF but were detected by one or more of the PA test methods, laboratories were requested to subculture the positives and send the resulting isolate for further identification.

Each subculture was checked for purity on nutrient agar and MacConkey agar. If more than one colony form was observed, further subcultures were made to ensure that identifications were made on pure cultures. Each isolate was lightly inoculated into 10 ml quarter strength Ringer's solution and a 1 μl loopful transferred to a further 10 ml of quarter strength Ringer's solution. This suspension was filtered through a cellulose membrane (0.45 μm nominal pore size). The membrane was placed on a filter paper pad soaked in MLSB and incubated at 30 °C for 4 hours followed by 37 °C for 14–16 hours. The presence of growth and the colour of the resulting colonies, if any, were noted.

3.3.4 Identification of strains forming yellow colonies on MLSB

Strains giving yellow colonies on MLSB were further identified to determine if they were *E. coli* or coliforms as defined by the old or new definitions. Tests for oxidase and gram stain on growth observed on the nutrient agar plate were carried out. The production of acid and gas from lactose in LPW at 37 °C and 44 °C, and the production of indole from tryptophan at 44 °C in TW were determined using the methods described in Report 71 (1983).

3.3.5 Identification of strains not forming yellow colonies on MLSB

Strains not producing yellow colonies on MLSB were examined by the gram stain and any that were gram-positive were not identified further. The remaining strains were tested for the presence of oxidase. All oxidase-negative isolates and a selection of oxidase-positive isolates were further identified using API20E or API20NE as appropriate.

3.4 Statistical methods

The data were entered and verified using the software package EPI-INFO. This was also used for data summaries and some statistical analyses. The proportion of samples showing presence of the relevant organisms by a particular method is measured as the number of positive results divided by the number of samples examined. The relative success of two different methods in their ability to demonstrate the presence of the organisms can be analysed by comparing the two proportions. The basic statistical method for comparing two proportions is the chi-squared test. This ignores the information that in the third phase of the study, both methods have been applied to subsamples from the same original sample. Therefore, if N samples were examined, N sets of paired results are obtained. It is more efficient for statistical purposes to tabulate the results taking into account this pairing and then applying McNemar's test or exact binomial probability to test the null hypothesis that the two methods find similar proportions of samples positive (Armitage 1971). An example is given in the next paragraph.

Paired comparisons were the fundamental criteria in meeting the specifications of the study—to compare the performance of PA tests with reference methods. For example, a comparison between MF and "kit A" from N water samples examines the results by pairing together the PA results from two sub-samples taken from each original sample which were used for the two methods. The results can be tabulated as:

		Kit A	
		negative	positive
MF	negative	k	r
	positive	s	m

A total of N samples was examined. Of these, k samples gave a negative result by both methods and m were positive by both methods. The discrepant results, r and s, are those which show whether or not one method was detecting more positive samples than the other. The null hypothesis is that both methods are equally efficient at detecting the organisms. The situation where one test is positive and the other is negative could occur by chance, and such situations are observed in most studies and experiments. They are especially likely in this study of water samples with small numbers of organisms. Even if the original sample is thoroughly mixed it is impossible to achieve uniform distribution, ie equal numbers of organisms in each sub-sample. The best that can be expected is random distribution with some inevitable variation between sub-samples (Tillett 1993). For example, if 600 ml of sample contains 12 coliforms and 100 ml is to be used for each of the 6 study methods, then the **average** number of coliforms per sub-sample will be 2 but the **actual** number may range from 0 to 4 or more due to random fluctuations. Thus some of the discrepant results in the table (r and s results) will be correct because one of the methods received a sub-sample with zero organisms. However, if one method is worse than the other at detecting organisms which are actually present then the value of r will become significantly larger than the value of s or **vice versa.**

McNemar's test statistic is equivalent to measuring the probability of tossing a coin (r+s) times and finding the difference between "heads" and "tails" as large as (r–s) (ie using the binomial distribution with parameter equal to 1/2). McNemar's test is used with larger numbers, but where the numbers are small then McNemar's test becomes inaccurate and so the exact two-tailed binomial probability was calculated using GLIM software (Baker and Nelder 1978).

The results are tabulated by laboratory. Comparisons of proportions positive are given by laboratory and heterogeneity demonstrated (ie the magnitude and direction of the differences between proportions differed significantly between laboratories). This meant that a single statistical model to describe test performance would be misleading without investigating geographical parameters and interactions. Although it is beyond the remit of the study to estimate performance at individual water sources, this heterogeneity in relative performance of methods with waters from different sources demonstrated that a detailed scrutiny of the data is essential. Therefore, Appendix B shows all results by individual water source, and statistically paired comparisons (using exact binomial probability) are shown for selected sources. Selection was made on the grounds of frequent sampling (at least 20) and giving the target low counts and thus intermittently positive results, defined as average positivity over all the methods of between 25% and 75%. Appendix B is presented in this booklet to provide a full picture of the results and to illustrate why overall conclusions (geographically) cannot be justified and why statistical findings need careful interpretation.

3.5 Results

Eight laboratories participated, examining 1409 samples. Over 52 water sources were used; for 22 sources, twenty or more samples were examined, and for 13 sources, between ten and nineteen samples. Most sources were PWS but there were also recreational waters and reservoirs. Details by laboratory and source are given in Appendix B.

Samples were collected between October 1993 and March 1994. Monthly totals together with numbers of samples presumptively positive by MF are shown in Table 3.

In the early months of the study, there were more positive samples than anticipated but the target result (whereby approximately half the samples were positive) was achieved.

3.5.1 Total coliform organism results

Action is triggered by presumptively positive MF results read the next day and so numbers of samples giving one or more yellow colonies by MF are compared in Table 4 with numbers of samples found positive by the kits at the recommended final time of reading.

Overall LMX and Colilert gave more positive results than MF; MMGM and Colitrace gave fairly similar numbers and PAB gave fewer positives. This is confirmed by the statistical analysis shown in Table 5.

These overall comparisons should be interpreted with caution. There was considerable variation between laboratories due to differences between water sources. The 95% confidence interval reflect an average difference for the samples in this study. The true differences for some water sources may be considerably greater.

3.5.1.1 Reaction time of kits

Four of the kits were read at an intermediate time as well as the recommended final time.

Of the 637 positive results from PAB at 48 hours, 360 (56.5%) were positive at 24 hours.

Of the 1037 positive results from LMX at 48 hours, 440 (42.4%) were positive at 24 hours.

Of the 766 positive results from Colitrace at 48 hours, 283 (36.9%) were positive at 24 hours.

Of the 935 positive results from Colilert at 28 hours, 680 (72.7%) were positive at 24 hours.

Thus the 1409 samples yielded presumptive positive results at the "next day" reading (ie 18 or 24 hours) in the following numbers:

797 (56.6%) by MF

360 (25.6%) by PAB

440 (31.2%) by LMX

283 (20.1%) by Colitrace

680 (48.3%) by Colilert

3.5.1.2 Confirmation of presumptive MF results

Table 6 shows the numbers of samples giving presumptive positive results, by laboratory. The third column shows the numbers of these samples for which at least one yellow colony confirmed as a coliform as defined by the old definition. The fourth column shows the number of samples where at least one yellow colony confirmed as a coliform as defined by the new definition, but none confirmed by the old definition. The fifth column shows the number of samples confirming as positive by either definition.

It was noted that there was a highly statistically significant variation geographically both in overall confirmation rates and in ratios of new to old definition confirmations. Laboratories 2 and 7 found proportionally more "new" coliform positive samples than other laboratories and, as will be seen later, their water sources were yielding lower rates of *E. coli* positive samples. These two laboratories had exceptionally low numbers of positive results from PAB.

3.5.1.3 Accuracy of the kit results

Ideally, it would have been appropriate to measure the sensitivity and specificity of these kits. However, it was not possible to achieve this according to the correct statistical definitions of the terms. It is impossible to distinguish between true and false negative results because of the random variation in numbers of organisms in the subsamples.

However, approximate sensitivity can be estimated by comparing numbers of positive results with those of presumptive MF results. Further information on accuracy is also available from comparisons with confirmed MF results and from the subcultures which were taken from positive test kits for a **selection** of samples which were negative by MF.

Table 7 shows that 144 (16.8%) of the 857 subcultures from test kits were found to be false positives. A summary of the organisms found is given in Table 8, and further discussion is given in section 3.5.4.3. The lowest false positive rates were observed with LMX and Colilert; the highest with Colitrace, the differences are statistically significant ($p<0.04$). However, the subcultures are a selection of positive test kits from samples where the MF result was negative. Therefore, the overall false positive results for the kits were likely to be lower than those shown in Table 7. Even these false positive rates would not account for the high numbers of samples found positive by LMX and Colilert in Table 5. Some of the false positive results (75 of 114) came from two individual water sources, again indicating that most waters yielded very few false positives by the kits. Of the 857 subculture results shown in Table 7, 518 (60%) showed coliforms as defined by the new definition.

3.5.1.4 Comparison with confirmed results

If Table 5 were repeated comparing the test kit results with the 709 confirmed positive MF results rather than the 797 presumptively positive MF results, then PAB would still give significantly fewer positive results (only 637); MMGM (773) and Colitrace (766) would give significantly more positive results, although these would include some false positives. LMX and Colilert would give highly significantly more positives than confirmed MF.

The result from MF gives an indication of what **might** have been in the corresponding sub-samples examined by the test kits. It should be remembered that the sub-samples can vary in content, even from a well mixed sample, both in numbers and types of organisms. For example, if a sample contained only two organisms—one of which was an "old" coliform as defined, and one a "new" coliform as defined - then this could lead to the correct but apparently contradictory result of MF positive as defined by the old definition, one test kit positive as defined by the new definition and all others negative. This is an extreme example but does illustrate that the results in Table 9, where confirmed MF results are compared with test kit results, are not expected to show exact correlation but only trends in agreement.

All five methods found proportionally few (4.2–7.4%) of their positive results (among the 88 samples) giving false positive results with MF. LMX and Colilert produced about 30% of their positives from samples which had given a negative MF result (no yellow colonies). Numerically and proportionally, PAB reported lower samples which had grown "new definition" coliforms only with MF.

Disparity between test kits is illustrated in Table 9 in that the highest positive numbers are reported using LMX in every row, and the lowest with PAB. However, some correlation with MF results is apparent from the consistent ranking of the row percentage figures (%) for every kit. The lowest positive percentage figures are for the first row (MF, negative) and the highest are for the third row (MF, positive as defined by old definition). Some test kits, especially LMX, were able to detect high numbers of positives from samples where MF was negative or false positive.

3.5.1.5 Presumptive counts from MF compared with kit results

Table 10 illustrates samples according to numbers of yellow colonies counted on MF. When the average count is low it is not unusual for a 100 ml sub-sample to contain no relevant organisms at all. Therefore many of the negative results are correct and should not be labelled "false negatives". However, as the average count increases most sub-samples would be expected to show evidence of coliform organisms. For example, if one 100 ml sub-sample yields 5 organisms by MF then the probability that another sub sample contains no organisms is only 0.03 (ie 3%).

All test kits show an increase in proportions of samples giving a positive result as the MF count increases. For several test kits, the discrepancies when counts are 5 or more are greater than the theoretical 3%, indicating that other factors are involved.

3.5.2 *E. coli* results

Three of the test kits in the study (LMX, Colitrace and Colilert) give presumptive "presence" results for *E. coli* if they react with a colour change and fluoresce. Presumptive positives by MF were all subjected to confirmatory tests for *E. coli*. Results are shown in Table 11

As shown in Table 12, overall, LMX and Colitrace found significantly more samples positive for *E. coli* than MF, and Colilert found a comparable number to MF.

These comparisons reflect average differences for the samples in this study but the true differences for some water sources appeared more extreme than seen here, and sometimes in significant contrast.

3.5.2.1 Reaction time of kits

Three kits were read at an intermediate time as well as the recommended final time.

Of the 351 positive LMX results at 48 hours, 263 (74.9%) were positive at 24 hours.

Of the 345 positive Colitrace results at 48 hours, 172 (49.9%) were positive at 24 hours.

Of the 287 positive Colilert results at 28 hours, 209 (72.8%) were positive at 24 hours.

Thus, although Colilert showed significantly more positive results for coliforms at 24 hours than any other test kit, see section 3.5.1.1, Colilert reported significantly fewer *E. coli* positives than LMX at 24 hours (209 compared with 263).

Thus the 1409 samples when read the "next day" (ie 18 or 24 hours) gave 797 (56.6%) presumptive positives for coliform by MF, see Table 4, but it was impossible to say which were presumptive *E. coli*. The three following kits were demonstrating some presumptive *E. coli* at this time:

263 (18.7%) by LMX

172 (12.2%) by Colitrace

209 (14.8%) by Colilert (which was also read at 28 hours).

3.5.2.2 Confirmed *E. coli* counts from MF compared with kit results.

Table 13 shows data for *E. coli* similar to that presented for presumptive counts in Table 10, see also section 3.5.1.5.

LMX and Colitrace reported more *E. coli* positive than Colilert at 4 of the 5 levels. For MF counts of 5 or more, results for all 3 test kits were close to the theoretical approximate deficit of 3% due to random variation between sub-samples.

3.5.3 Geographical variation

Tables 4 and 11 demonstrate highly significant heterogeneity between laboratories when pairs of methods are compared - both for coliforms (Table 4) and *E. coli* (Table 11). Results for individual water sources are shown in Appendix B.

3.5.4 Identifications

A **proportion** of positive tests were sub-cultured when MF failed to detect any coliforms in a sample. These subcultures yielded 857 isolates of which 713 (83.2%) were identified as members of the Enterobacteriaceae, ie they would conform as coliforms as defined by the new definition. The remaining 144 (16.8%) could be considered false positives. A total of 195 (27.3%) of the 713 Enterobacteriaceae were coliforms as defined by the old definition and the remaining 518 (72.7%) would be classified as coliforms as defined by the new definition only.

3.5.4.1 Identifications of Enterobacteriaceae (coliforms)

Strains growing on MLSB as yellow colonies—A total of 348 (48.8%) of the strains of Enterobacteriaceae were capable of growth as yellow colonies on membranes impregnated with MLSB. The failure of MF to detect these strains in the original sample was therefore likely to be due to chance (ie there were no relevant organisms in the sub-sample examined by MF). In some instances it is possible that the recovery of these strains on MLSB by MF was less efficient than the recovery in the liquid PA test media, however this was not determined.

The identifications of the strains capable of being detected by MF technique are shown in Table 14. A total of 191 (54.9%) of these 348 strains were coliforms as defined by the old and new definitions, and 157 (45.1%) were coliforms as defined only by the new definition. Of the 166 strains that were coliforms as defined by both definitions and were fully identified, 164 (98.8%) proved to be *E. coli*. A total of 32 of the strains that were coliforms as defined by the new definition were fully identified, and most of these were *Enterobacter* species.

Strains not growing on MLSB as yellow colonies—A total of 365 (51.2%) of the 713 isolates of Enterobacteriaceae did not grow on MLSB or produced colonies that were not yellow. These isolates therefore represent the organisms detectable by the PA tests but not by MF. As might be anticipated, the majority (361, 98.9%) were coliforms as defined by the new definition only, but 4 were strains of *E. coli* that confirmed to the old definition but appeared to be unable to grow on MLSB. The identifications of the strains according to API20E are shown in Table 15. Not all strains could be adequately classified to the species level. The most common species were *Cit. freundii* and *Ent. intermedius* and 27 (7.4%) were classified as *Y. enterocolitica*.

3.5.4.2 Identification by test method

The identifications of the strains isolated from the individual test methods are summarised in Table 16. The overall spectrum of organisms detected was similar for all of the tests although the relative proportions differed from test to test. Proportionately more strains of *Cit. freundii* were isolated from Colilert (11%) and LMX (12%). Similarly, *Ent. intermedius* was less commonly isolated from MMGM (9%) and PAB (3%) than from Colilert (16%), Colitrace (16%) and LMX (13%). *Hafnia alvei* was not isolated from Colilert but was from all of the other tests and most commonly from LMX (5%). There were isolates of three different *Serratia* species from each of LMX, PAB, and Colilert but only 2 isolates of *Ser. fonticola* were made from Colitrace and no strains of *Serratia* were isolated at all from MMGM. *Yersinia enterocolitica* was isolated from all of the test kits.

3.5.4.3 Presumptive false positives

The identifications of the strains that proved not to be members of Enterobacteriaceae are given in Table 8. Of the 144 strains, only 7 (4.9%) were gram-positive including 5 enterococci and 2 *Bacillus* species. Of the remaining 137 gram negative isolates, representing those that did not grow as yellow colonies on MLSB, 56 (40.9%) were identified further. The majority, ie 46 out of 56 (82.1%) were *Aeromonas* species, 5 (8.9%) were *Pseudomonas fluorescens* and the remainder were other pseudomonads.

Aeromonas species were isolated from all of the media. Pseudomonads (*Pseudomonas*, *Chryseomonas* and *Xanthomonas* species) were isolated from all of the media except LMX. *Enterococci* were only isolated from PAB.

The numbers of presumptive false positives by water source are shown in Table 17.

3.6 Discussion

For phase three of the study, the eight laboratories selected sources that, from previous experience, when tested by MF, would have been expected to yield samples containing less than 5 coliforms/100 ml such that they would be negative on average in about 50% of samples. All of the sources were non-chlorinated and most of them were PWS.

The benefit of being able to study widely geographically dispersed sources was confirmed by the considerable variation found between laboratories and sources as is illustrated by the results in Table 6. From the MF results it can be seen that this difference applied to the proportion of presumptive coliforms that confirmed according to both the new and old definitions, and the incidence of *E. coli*. It can also be seen from the test kit results where, relative to MF, the incidence of discrepant results and presumptive false positive isolates varied with source (see Appendix B). Thus many of the overall conclusions should be interpreted with caution.

With regard to the confirmation of presumptive coliforms detected by MF, much of the variation in confirmation between laboratories can apparently be accounted for by the incidence of *Aeromonas* species being much higher in some sources than others. In particular, laboratory 2, the laboratory with the lowest confirmation rate also had a source, a spring water, which yielded the highest number of isolates of *Aeromonas* from the presumptively false positive PA tests which were selected for sub-culturing.

In order to discuss the potential value of PA tests relative to MF for routine testing of water samples it is necessary to consider the purposes for which the testing of water is undertaken. The detection of coliforms is required for regulatory purposes and also for operational monitoring. The detection of coliforms, in general, also has some public health significance but for this, the detection of *E. coli* is essential. The results of the tests are considered in relation to:

(1) satisfying regulatory requirements for determining coliforms;
(2) the detection of *E. coli*; and
(3) the detection of coliforms for operational management

3.6.1 The detection of coliforms

In practice, to ensure the most rapid control of any potential failures in water quality, water companies will normally react to presumptive coliform and *E. coli* counts rather than wait for confirmation which may take a further 24 hours. MF will usually provide a presumptive result for coliforms and *E. coli* within 18 hours, although membrane filters can be examined much earlier than this, for example 12 hours, especially where moderate or high contamination is suspected. Thus for a PA test to be of benefit it needs to provide a comparable result **within the same time scale.**

After 24 hours incubation, all of the tests gave considerably fewer presumptive positives than MF. Only Colilert gave a proportion (48%) that approached that found positive by MF (57%). By 28 hours, the maximum recommended incubation for Colilert, the proportion of positives (66%) exceeded that found by MF. However, in

order to determine the ability of the tests to detect coliforms to the level required for regulatory purposes (1 coliform/100 ml) waters were examined with low counts of coliforms. In practice, when present, the numbers of coliforms would frequently exceed this level and the PA tests might therefore be expected to give a positive response earlier.

PAB, MMGM and Colitrace detect coliforms by the production of acid from lactose and would therefore be expected to detect the same kinds of coliforms as MF. Thus after incubation for the full recommended time one might expect the test kits to yield a similar number of positives if they are equally efficient. MMGM and Colitrace did give a similar proportion of positives but PAB produced significantly less.

Colilert and LMX indicate the presence of coliforms by detecting ß-galactosidase and therefore will detect not only lactose fermenters but also coliforms that produce ß-galactosidase without being able to ferment lactose to acid. Thus, assuming such organisms are present and the tests are as effective as MF in detecting the lactose fermenters it might be expected that they would produce more positives. This, indeed, was the case and both Colilert and LMX detected significantly more positives after the full recommended incubation times of 28 and 48 hours respectively. Similarly, the identification of the isolates from selected positive kits indicates that Colilert and LMX yielded a higher proportion of coliforms as defined by the new definition (73% and 66% respectively, Table 7) than the other kits.

A higher incidence of presumptive positives might be explained by a higher incidence of false positives. Subcultures from a **selection** of tests displaying discrepant results relative to MF were studied. The results give an over estimate of false positive rates because concordant samples were not sub-cultured. However, the results illustrate the relative size of the problem between kits. The subcultures yielded a number of isolates that were not coliforms. Colilert and LMX showed the lowest incidence of such "false-positive" organisms. However the isolation of such an organism from a test does not necessarily mean that the test was giving a false positive reaction for there may have been a coliform present in sufficient numbers to generate a positive response while still being only a minor proportion of the bacterial population and therefore less likely to be detected on subculture. Indeed for many of these "false positive" isolates other sub-samples of the same sample had grown confirmed coliforms in other PA tests. This is another reason why the figures for presumptive false positive isolates will be greater than the number of true false positive tests. Even if they were all true false positives it is seen that they would not account for the high numbers of samples found positive by Colilert and LMX.

As the coliform count increases, the probability of a sub-sample having no coliforms in it by chance decreases. When the numbers reach 5 or more, on average, the chance of another sub-sample containing no coliforms decreases to 0.03 so that any test method would be expected, on average, to have about 97% of sub-samples positive. The results in Table 10 show this was true for LMX and Colilert, but MMGM and Colitrace had 88% and PAB only 86%. This suggests that these three tests are giving some false negatives when the average sub-sample count exceeds 4/100 ml. This result was not expected but might be explained by the presence of other non-coliform organisms in the samples being able to grow and compete with the coliforms. This may delay or even prevent the growth of the coliforms and so delay or prevent the test giving a positive response.

From the above discussion it may be concluded that Colilert and LMX both detect more coliforms than MF when incubated for the maximum recommended times of 28 and 48 hours respectively. MMGM and Colitrace both gave comparable results to the MF method when incubated for the full 48 hours, but PAB was somewhat less effective. Thus, if one is only interested in the final presumptive result MMGM, Colitrace, Colilert and LMX would all give comparable or higher numbers of positives to MF. In contrast, the time to a presumptive positive is faster by MF at the levels of contamination studied. Therefore, the use of these tests for routine operational management will depend on the level of contamination that is likely to be of concern and the speed with which it needs to be detected.

3.6.2 Detection of *E. coli*

From a public health point of view, it is imperative to be able to detect potential faecal pollution of a water supply as soon as possible. In this context, this means the ability to detect *E. coli* quickly and, in addition, with a sensitivity of one *E. coli*/100 ml. Three of the PA tests examined were designed to indicate the presence of *E. coli* by detecting the presence of β-glucuronidase activity which, within Enterobacteriaceae, is highly specific for *E. coli*. In phase 2 of the study using simulated water samples with low numbers of coliforms and *E. coli*, LMX and Colilert produced a statistically significantly lower proportion of positives than MF for *E. coli*, although the kits were comparable for *Ent. cloacae* and *K. pneumoniae*. (See Table 2). Colitrace gave a slightly higher proportion of positives for *E. coli* that was not statistically significant. For *K. pneumoniae*, Colitrace gave a statistically significantly higher proportion of positives than MF. In phase 3 of the study, after appropriate full incubation periods, LMX and Colitrace yielded significantly more samples positive for *E. coli* than MF and Colilert detected a comparable number, see Table 11. To detect *E. coli* by MF requires more than 1 day because of the confirmation step, but all of test kits could potentially provide a result earlier, provided there is sufficient growth. The tests were examined after 24 hours and LMX gave significantly more positives than Colilert and Colitrace after 24 hours even though the recommended incubation time is 48 hours. After 24 hours, LMX produced 74.9% of the positives generated after 48 hours and the number of positives at 24 hours represented 87% of the number of confirmed positives by MF after 48 hours, see section 3.5.2.1.

When the response of the kits was examined in relation to the number of *E. coli* detected by MF it was evident that LMX and Colitrace were detecting more positives than Colilert for 4 of the 5 levels of contamination examined, see Table 13.

With Colitrace, fluorescence was sometimes recorded in the absence of acid production. It is possible that some other bacteria present in the sample possessed β-glucuronidase without being able to ferment lactose.

The results of this study, being the first study of PA test kits which has concentrated its assessment of methods using only raw waters containing low numbers of coliforms and *E. coli*, indicate that there is no single test kit that is considered appropriate for every location.

3.6.3 Identifications

As was anticipated, the use of the new definition of coliforms generated a number of extra positives by MF and the proportion of extra positives varied from source to source.

All of the tests kits yielded a variety of coliforms as defined by the new definition from the selected subcultures, but, as expected, the numbers of these were much higher for LMX and Colilert in which coliforms are detected by the production of ß-galactosidase as opposed to the production of acid from lactose. It was noted that even media which detected lactose fermentation, some isolates produced pink or colourless colonies on MLSB. Although some may have come from mixed cultures where the minority organism produced acid from lactose, it is also possible that some produce sufficient acid in the liquid media for them to be detected but insufficient to produce yellow colonies on MLSB.

Although designed primarily for the identification of Enterobacteriaceae, the API20E database has been generated largely from information gathered from clinical isolates and consequently may not identify environmental isolates to the species level as reliably as clinical ones. Unfortunately, there are no identification systems with a comparable data base generated from environmental isolates. Nonetheless, the spectrum of coliforms isolated from LMX and Colilert was much as expected. All of the genera or species that are listed in Table 1 as being coliforms by either definition and occurring commonly in water were detected in the samples studied. The most common identifications were *E. coli*, *Cit. freundii* and *Ent. intermedius*.

The relatively frequent identification of *Ent. intermedius* is of interest because it is listed as being a lactose fermenter. The majority of these isolates grew as pink colonies on MLSB but many were isolated from MMGM and Colitrace suggesting that in these liquid media the lactose fermentation is successfully expressed.

According to Farmer and Kelly (1991) about 50% of *Cit. freundii* strains are lactose fermenters but all produce β-galactosidase. Thus, in contrast to *Ent. intermedius* most of the strains of *Cit. freundii* were isolated from LMX and Colilert presumably because they were unable to produce acid from lactose but possessed β-galactosidase.

The next most common group consisted of strains that, using API20E, could not be clearly allocated to any species but had characters intermediate to the genera *Buttiauxella* and *Enterobacter.*

Numerically *Y. enterocolitica* was the next most common species and was found in a wide range of sources. This observation is of some public health interest since *Y. enterocolitica* can be pathogenic. Although all strains produce β-galactosidase the production of acid from lactose, according to Farmer and Kelly (1991), is only found in 5% of strains. Thus it was not surprising that *Y. enterocolitica* was isolated most commonly from LMX but it was unexpected that they would be detected by MMGM, PAB and Colitrace. These identifications may be correct and the strains may represent lactose fermenting variants. Alternatively, they may be other species of *Yersinia* that ferment lactose and the failure to identify them may reflect inadequacies in the API20E data base or the water sub-samples may have contained more than one coliform and only the non lactose fermenter was isolated on sub-culturing the lactose containing test kits.

Since LMX and Colilert detect β-galactosidase which is more common than lactose fermentation among the new coliforms, it was not surprising that the numbers of new coliforms detected by Colilert and LMX greatly exceeded the numbers isolated from the other media. In general, the spectrum of organisms detected by all of the test kits was similar but the low isolation rate of *Y. enterocolitica* and *H. alvei* from Colilert was surprising when compared to the results for LMX.

The spectrum of species and genera identified in this study was biased by the selection of tests sub-cultured, and does not necessarily represent the range of organisms that can grow in the test kits or the incidence of the individual groups or species in the original water samples. The collection does, however, represent the range of organisms that might be expected to be detected by these PA methods in addition to those that would normally be detected by MF. It can be seen from Tables 14 and 15, with the exception of *E. coli*, the vast majority of discrepant results were caused by strains that identified as coliforms as defined by the new definition and would not be detected by MF. In order to determine the complete range of organisms detected by the individual test methods it would have been necessary to isolate and identify strains from representatives of all positive tests. This was not possible within the remit and resources of the current work but in view of the general concordance between the results of the PA kits and MF it is unlikely that the spectrum of coliforms as defined by the old definition detected by the kits is significantly different to that detected by MF.

3.6.4 Recovery of injured organisms

Both MMGM and Colitrace in general gave comparable results to MF for both coliforms and *E. coli* and LMX gave more positive coliform and *E. coli* results. There was, nonetheless, some variation between sources but this is probably also true for MF. In this study, only non-chlorinated waters were examined as preliminary work indicated that it was impractical to test all of the kits in parallel. However many of the strains, particularly the *E. coli*, detected in the samples examined were likely to be stressed in other ways. In addition, the suspensions used in phase 2 of the study were stabilised with borate which also induces some injury. In phase 2, LMX and Colilert gave significantly lower recoveries than MF for the *E. coli* strain examined. In phase 3 of the study, for *E. coli*, Colilert reported fewer positives than the other kits and LMX generally more positives.

The performance of MMGM in recovering chlorine damaged organisms is known and can be considered satisfactory since it has been in use for many years as the medium used for the reference MPN method. In general, MMGM is believed to give slightly better recovery of injured organisms than MF using MLSB. Colitrace would be expected to behave similarly to MMGM.

3.6.5 The use of PA tests for compliance monitoring

If a PA test performs at least as well as MF at detecting coliforms and *E. coli* then its use for the compliance monitoring of drinking water would afford the same degree of consumer protection. However, there would be no quantitative information. In principle, there appears to be no public health risk attached to the use of PA test kits and, provided recovery of chlorine damaged organisms can be shown to be satisfactory, the use of a test kit may improve the ability to detect more rapidly any potential risks to health. The variation displayed between sources suggests that it may be necessary to validate the new test kit against the local flora at each source before deciding to use it.

Many of the sources examined were PWS and the results indicate PA tests may be particularly useful for monitoring these sources since they can be easily inoculated in the field.

3.6.6 The use of PA tests for process control

To provide an advantage over MF, a PA test kit needs to enable at least as quick a response as MF without reducing the chances of detecting a risk to public health. In terms of detecting *E. coli*, LMX in particular, may offer an improvement but at the risk of generating more coliform failures after 48 hours. The only test with as quick a response as MF for detecting coliforms was Colilert but this did not perform as well at detecting *E. coli*. In practice, however, the response to a positive coliform count may be related to the count detected. High counts of coliforms have not been examined in this study but it is likely that these would give an earlier response in the PA tests than 24 hours. In terms of detecting *E. coli* which requires an immediate response, LMX and Colilert would both give a response inside 24 hours in most instances. Thus, in practice, the slow response of some of these kits to low numbers of coliforms may not be a significant problem and the chances of detecting heavy contamination or *E. coli* earlier than MF may be an advantage but the performance of the test may have to be validated for each source.

The ideal PA test for process control would detect coliforms and *E. coli* at least as well as MF and give the result for both within 24 hours. None of the tests examined in this study satisfied all of these criteria for coliforms, but LMX approached it for *E. coli*.

Table 1 The genera and species within the family Enterobacteriaceae and their classification according to the new and old definition of coliforms

Genus or species within the family Enterobacteriaceae	Coliform as defined by		Reason for difference, if any, in classification by old and new definition
	New Definition (posessing b-galactosidase)	Old definition (acid and gas)	
Budvicia aquatica	yes	53% yes	47% do not produce gas at 37 °C and 13% do not ferment lactose
Buttiauxella agrestis	yes	yes	
Cedecea spp.	yes	some	lactose fermentation differs from strain to strain
Citrobacter spp.	yes	20–50% yes	lactose fermentation variable, β-galactosidase positive
Edwardsiella spp.	no	no	
Enterobacter spp.	yes	yes (few no)	*Ent. agglomerans*, *Ent. gergoviae*, *Ent. taylorae* and *Ent. amnigenus* are lactose variable but normally possess β-galactosidase
E. coli	yes	yes	
E. adecarboxylata	no	no	
E. blattae	no	no	
E. fergusonii	83%	no	lactose negative but 83% have β-galactosidase
E. hermanii	yes	45% yes	45% lactose fermenters but 98% have β-galactosidase
E. vulneris	yes	no	85% lactose negative but all have β-galactosidase
Ewingella spp.	85% yes	no	no gas production although lactose fermented
Hafnia spp.	yes	no	lactose negative but have β-galactosidase
K. ozaenea	80% yes	70% no	70% lactose negative but 80% have β-galactosidase
K.. rhinoscleromatis	no	no	
Other *Klebsiella* spp.	yes	yes	
Kluyvera spp	yes	yes	
Leminorella	no	no	
Moellerella spp.	yes	yes	
Morganella spp.	no	no	
Obesumbacteria spp.	no	no	
Pragia	no	no	
Proteus spp.	no	no	
Providencia spp.	no	no	
Rhanella aquatilis	yes	yes	
Salmonella spp	no	no	
Serratia spp.	yes	varies	have β-galactosidase but lactose fermentation varies from species to species
Shigella sonnei	yes	no	have β-galactosidase but lactose negative
Other *Shigella* spp.	no	no	
Tatumella spp.	no	no	
Yersinia enterocolitica	yes	no	lactose negative but have β-galactosidase
Xenorhabdus spp.	no	no	

Table 2 Summary of phase 2 results

Organism (200 batches for each organism)	Test	Proportion of samples positive	Difference (d) compared with proportion positive by MF	Approximate 95% confidence interval for d, the difference in proportions
E. coli	MF	.700		
	MPN	.715	+0.015	–0.07 to +0.10
	PAB	.600	–0.100	–0.19 to +0.01
	LMX	.455	–0.245	–0.34 to –0.15*
	Colitrace	.730	+0.030	–0.06 to +0.12
	Colilert	.475	–0.225	–0.32 to –0.13*
Ent. cloacae	MF	.725		
	MPN	.750	+0.025	–0.06 to +0.11
	PAB	.770	+0.045	–0.04 to +0.13
	LMX	.740	+0.015	–0.07 to +0.10
	Colitrace	.740	+0.015	–0.07 to +0.10
	Colilert	.780	+0.055	–0.03 to +0.10
K. pneumoniae	MF	.540		
	MPN	.615	+0.075	–0.02 to +0.17
	PAB	.525	–0.015	–0.11 to +0.08
	LMX	.585	+0.045	–0.05 to +0.14
	Colitrace	.660	+0.120	+0.02 to +0.22**
	Colilert	.610	+0.070	–0.03 to +0.17

* indicates a statistically significantly lower proportion of samples positive (ie the 95% confidence interval does not include zero).
** indicates a statistically significant higher proportion of sample positive (ie the 95% confidence interval does not include zero).

Table 3 Presumptive MF coliform results by month

Month	Total Samples	Samples giving yellow colonies
Oct 93	16	14 (87.5)
Nov 93	256	162 (63.3)
Dec 93	282	186 (66.0)
Jan 94	253	139 (54.9)
Feb 94	408	189 (46.3)
Mar 94	194	107 (55.2)
Total	1409	797 (56.6)

Figure in brackets indicates percentages (%).

Table 4 Samples showing the presence of coliform organisms

Lab No	Total samples examined	Positive Results					
		MF* (20 hr)	MMGM (48 hr)	PAB (48 hr)	LMX (48 hr)	Colitrace (48 hr)	Colilert (28 hr)
1	230	116 (50.4)	120 (52.2)	108 (47.0)	155 (67.4)	137 (59.7)	135 (58.7)
2	202	98 (48.5)	60 (29.7)	20 (9.9)	158 (78.2)	63 (31.2)	149 (73.8)
3	204	159 (77.9)	169 (82.8)	133 (65.2)	183 (89.7)	153 (75.0)	177 (86.8)
4	200	138 (69.0)	150 (75.0)	178 (89.0)	181 (90.5)	146 (73.0)	137 (68.5)
5	219	81 (37.0)	117 (53.4)	104 (47.5)	155 (70.7)	108 (49.3)	148 (67.6)
6	123	79 (64.2)	55 (44.7)	54 (43.9)	83 (67.5)	61 (49.6)	79 (64.2)
7	198	107 (54.0)	87 (43.9)	25 (12.6)	101 (51.0)	81 (40.9)	97 (49.0)
8	33	19 (57.6)	15 (45.5)	15 (45.5)	21 (63.6)	17 (51.5)	13 (39.4)
Total	1409	797 (56.6)	773 (54.9)	637 (45.2)	1037 (73.6)	766 (54.4)	935 (66.4)

* presumptive MF.
Figures in brackets indicate percentages (%).
Percentages are of total samples examined by that laboratory or, in the final row, of the overall total.

Table 5 Overall comparison of PA tests with MF (1409 Samples)

Test	Proportion of samples positive	Difference (d) compared with proportion positive by MF*	p-value§	Approximate 95% confidence interval for d, the difference in proportions
MF	0.566			
MMGM	0.549	–0.017	0.2	–0.05 to +0.02
PAB	0.452	–0.114	<0.00001	–0.15 to –0.08#
LMX	0.736	+0.170	<0.00001	+0.14 to +0.21@
Colitrace	0.544	–0.022	0.1	–0.06 to +0.01
Colilert	0.664	+0.098	<0.00001	+0.06 to +0.13@

* 0.566 (ie 56.6%) of samples were presumptively positive by MF.
§ using McNemar's test for paired comparisons.
# indicates a statistically significantly lower proportion of samples positive (ie the 95% confidence interval does not include zero).
@ indicates a statistically significant higher proportion of sample positive (ie the 95% confidence interval does not include zero).

Table 6 Confirmation of presumptively positive MF results

Lab No	Presumptive positive samples	Confirmed positive as defined by old definition (acid + gas)	Confirmed positive as defined by new definition only (ß-galactosidase)	Confirmed positive as defined by both definitions
1	116	72 (62.1%)	30 (25.9%)	102 (87.9%)
2	98	21 (21.4%)	44 (44.9%)	65 (66.3%)
3	159	140 (88.1%)	13 (8.2%)	153 (96.2%)
4	138	114 (82.6%)	10 (7.2%)	124 (89.9%)
5	81	66 (81.5%)	14 (17.3%)	80 (98.8%)
6	79	72 (91.1%)	6 (7.6%)	78 (98.7%)
7	107	50 (46.7%)	40 (37.4%)	90 (84.1%)
8	19	15 (78.9%)	2 (10.5%)	17 (89.5%)
Total	797	550 (69.0%)	159 (19.9%)	709 (89.0%)

Table 7 Results of subcultures from positive kits

	MMGM	PAB	LMX	Colitrace	Colilert	Total
Number of samples sub-cultured	113 (100%)	85 (100%)	276 (100%)	142 (100%)	241 (100%)	857 (100%)
Number confirming as coliforms by old definition*	29 (26.7%)	28 (32.9%)	55 (19.9%)	44 (31.0%)	39 (16.2%)	195 (22.8%)
Number confirming by new definition	62 (54.9%)	43 (50.6%)	183 (66.3%)	54 (38.0%)	176 (73.0%)	518 (60.4%)
Presumptive false positives# (ie other organisms)	22 (19.5%)	14 (16.5%)	38 (13.8%)	44 (31.0%)	26 (10.8%)	144 (16.8%)

* including typical *E.coli.*
Figures in brackets indicate percentages (%).
these percentages are overestimates—see section 3.5.1.3. See also Table 17.

Table 8 Identifications according to API20E or API20NE of strains isolated from presumptive false positive tests

Identification	Number of isolates	Percentages %
Aeromonas hydrophila/caviae	41	28.5
Aeromonas salmonicida	1	0.7
Aeromonas sobria	3	2.1
Aeromonas sp	1	0.7
Chryseomonas luteola	1	0.7
Enterococci	5	3.5
Gram positive bacillus	2	1.4
Oxidase positive, gram negative	80	55.6
Pseudomonas fluorescens	5	3.5
Pseudomonas putida	1	0.7
Xanthomonas maltophilia	3	2.1
API-Unidentified	1	0.7
Total	144	100.0

Table 9 MF coliform confirmation and corresponding PA results

MF	Number of samples	Positive kit results				
		MMGM	PAB	LMX	Colitrace	Colilert
Negative	612 (100)	173 [22.4] (28.3)	143 [22.4] (23.4)	313 [30.2] (51.1)	165 [21.5] (27.0)	268 [28.7] (43.8)
False positive (no colony confirmed)	88 (100)	42 [5.4] (47.7)	27 [4.2] (30.7)	77 [7.4] (87.5)	51 [6.5] (58.0)	66 [7.1] (75.0)
Positive as defined by old definition	550 (100)	459 [59.4] (83.5)	398 [62.5] (72.4)	504 [48.6] (91.6)	441 [57.6] (80.2)	475 [50.8] (86.4)
Positive as defined by new definition	159 (100)	99 [12.8] (62.3)	69 [10.8] (43.4)	143 [13.8] (90.0)	109 [14.2] (68.6)	126 [13.5] (79.2)
Total	1409	773 [100]	637 [100]	1037 [100]	766 [100]	935 [100]

Figures in brackets are percentages.
Percentage figures in square brackets [ ] gives the proportion of the column total which fall in the MF result category shown for that row. The percentage figures in normal brackets ( ) give the proportion of the row total and shows the proportion of samples in the MF result category which gave a positive result by the test kit named at the head of the column.

Table 10 Presumptive MF coliform counts and corresponding PA results

MF colony count	Number of samples	Number of the samples which were positive by:				
		MMGM	PAB	LMX	Colitrace	Colilert
0	612(100)	173(28.3)	143(23.4)	313(51.1)	165(27.0)	268(43.8)
1	313(100)	191(61.0)	148(47.3)	271(86.6)	200(63.9)	224(71.6)
2	164(100)	130(79.3)	101(61.6)	150(91.5)	131(79.9)	143(87.2)
3–4	160(100)	139(86.9)	108(67.5)	150(93.8)	129(80.6)	146(91.3)
5 or more	160(100)	140(87.5)	137(85.6)	153(95.6)	141(88.1)	154(96.3)

Figures in brackets are percentages (%).

Table 11 Confirmed and presumptive results for *E. coli* by laboratory

Lab No	Total samples examined	*E.coli* confirmed from		Presumptive *E.coli*	
		MF	LMX	Colitrace	Colilert
1	230	39 (17.0)	73 (31.7)	82 (35.7)	57 (24.8)
2	202	2 (1.0)	4 (2.0)	3 (1.5)	4 (2.0)
3	204	48 (23.5)	54 (26.5)	54 (26.5)	55 (27.0)
4	200	96 (48.0)	110 (55.0)	110 (55.0)	101 (50.5)
5	219	56 (25.6)	46 (21.0)	47 (21.0)	32 (14.6)
6	123	32 (26.0)	34 (27.6)	19 (15.4)	26 (21.1)
7	198	18 (9.1)	19 (9.6)	17 (8.6)	11 (5.6)
8	33	11 (33.3)	11 (33.3)	13 (39.4)	1 (3.0)
Total	1409	302 (21.0)	351 (24.9)	345 (24.5)	287 (20.4)

Figures in brackets are percentages (%).
Percentages are of total samples examined by that laboratory or, in the final row, of the overall total of 1409.

Table 12 Overall comparison of *E.coli* results

Test	Proportion of samples positive	Difference (d) compared with proportion positive by MF*	p-value#	Approximate 95% confidence interval for d, the difference in proportions
LMX	0.249	+0.035	0.004	+0.012 to +0.058**
Colitrace	0.245	+0.031	0.01	+0.008 to +0.054**
Colilert	0.204	–0.011	0.2	–0.033 to +0.012

* 0.214 (ie 21.4%) of samples gave confirmed *E. coli* by MF.
# using McNemar's test for paired comparisons.
** indicates a statistically significant higher proportion of samples positive (ie the 95% confidence interval does not include zero).

Table 13 *E. coli* counts by MF and corresponding PA results

MF colony count	Number of samples	Number of the samples positive for *E. coli* by:		
		LMX	Colitrace	Colilert
0	1107	164 (14.8)	159 (14.0)	126 (11.4)
1	137	57 (41.6)	63 (46.0)	50 (36.5)
2	68	52 (76.5)	43 (63.2)	37 (54.4)
3–4	50	32 (64.0)	35 (70.0)	28 (56.0)
5 or more	47	46 (97.9)	45 (95.7)	46 (97.9)

Table 14 Identification of strains of Enterobacteriaceae isolated from positive kits and capable of growth on MLSB as yellow colonies

Identification	Number with coliform definition		Total
	Old and New	New Only	
But. agrestis	1	0	1
Cit. freundii	0	4	4
Ent. aerogenes	0	5	5
Ent. agglomerans	0	2	2
Ent. cloacae	0	2	2
Ent. intermedius	0	8	8
Ent. sp	0	1	1
E. coli	164	1*	165
Enterob/Buttiauxella	0	2	2
K. pneumo. pneumo	1	0	1
New coliform**	0	125	125
Old coliform**	25	0	25
Prov. sp	0	1	1
Ser. fonticola	0	2	2
Y. enterocolitica	0	4	4
TOTAL	191	157	348

* Atypical *E. coli.*
** Not identified further.

Table 15 Identifications of strains of Enterobacteriaceae isolated from positive kits and not able to grow on MLSB as yellow colonies (ie coliforms as defined by the new definition that would be detected by PA tests but not by MF)

Identification	Frequency	Percentage (%)
But. agrestis	8	2.2
Cit.div.amalonat	2	0.5
Cit. freundii	60	16.4
Ent. aerogenes	11	3.0
Ent. agglomerans	5	1.4
Ent. amnigenus	19	5.2
Ent. cloacae	4	1.1
E. fergusonii	1	0.3
Ent. intermedius	83	22.7
Enterob/Buttiauxella	33	9.0
Erwi.nigrifluens	2	0.5
E.coli 1*	7	1.9
E.coli 2*	5	1.4
E. vulneris	19	5.2
H. alvei	17	4.7
K. pneumo.ozaenae	6	1.6
K. pneumo.pneumo	2	0.5
K. pneumo.rhino.	2	0.5
New coliform	2	0.5
Old coliform	2	0.5
Rahn. aquatilis	17	4.7
Ser. fonticola	18	4.9
Ser. liquefaciens	5	1.4
Ser. plymuthica	5	1.4
Ser. sp.	1	0.3
Y. enterocolitica	27	7.4
API- unidentified	2	0.6
Total	365	99.8

* Atypical strains of *E. coli*

Table 16 Identification of strains of Enterobacteriaceae isolated from the individual tests

Identification	Colilert		MMGM		PAB		Colitrace		LMX		Total	
But. agrestis	5	(2)	1	(1)	0		1	(1)	2	(1)	9	(1)
Cit. div. amalonat	1	(1)	0		0		0		1	(<1)	2	(<1)
Cit. freundii	24	(11)	4	(4)	4	(6)	4	(4)	28	(12)	64	(9)
Ent. aerogenes	6	(3)	2	(2)	3	(4)	0		5	(2)	16	(2)
Ent. agglomerans	1	(1)	1	(1)	1	(1)	1		3	(1)	7	(1)
Ent. amnigenus	10	(5)	1	(1)	1	(1)	2	(2)	5	(2)	19	(3)
Ent. cloacae	1	(1)	3	(3)	1	(1)	0		1	(<1)	6	(1)
E. fergusonii	0		0		0		0		1	(<1)	1	(<1)
Ent. intermedius	34	(16)	8	(9)	2	(3)	16	(16)	31	(13)	91	(13)
Ent. sp	0		0		0		1	(1)	0		1	(<1)
Enterob/Buttiauxella	15	(7)	7	(8)	1	(1)	2	(2)	10	(4)	35	(5)
Erwi. nigrifluens	1	(1)	1	(1)	0		0		0		2	(<1)
E. coli (typical)	31	(14)	24	(26)	22	(31)	37	(38)	45	(19)	159	(22)
E.coli (atypical)	7	(3)	1	(1)	0		2	(2)	8	(3)	18	(3)
E. vulneris	8	(4)	1	(1)	1	(1)	0		9	(4)	19	(3)
H. alvei	0		1	(1)	2	(3)	3	(3)	11	(5)	17	(2)
K. pneumo. ozaenae	3	(1)	0		0		1	(1)	2	(1)	6	(1)
K. pneumo. pneumo	1	(1)	1	(1)	0		0		1	(<1)	3	(<1)
K. pneumo. rhino.	1	(1)	1	(1)	0		0		0		2	(<1)
New coliform	45	(21)	23	(25)	12	(17)	10	(10)	37	(16)	127	(18)
Old coliform	5	(2)	3	(3)	6	(9)	6	(6)	7	(3)	27	(4)
Prov. sp	0		0		0		0		1	(<1)	1	(<1)
Rahn. aquatilis	4	(2)	4	(4)	3	(4)	2	(2)	4	(2)	17	(2)
Ser. fonticola	4	(2)	0		4	(6)	2	(2)	10	(4)	20	(3)
Ser. liquefaciens	1	(1)	0		1	(1)	0		3	(1)	5	(1)
Ser. plymuthica	4	(2)	0		0		0		1	(<1)	5	(1)
Ser. sp.	0		0		1	(1)	0		0		1	(<1)
Y. enterocolitica	3	(1)	4	(4)	6	(9)	6	(6)	12	(5)	31	(4)
API- Unidentified	0		0		0		2	(2)	0		2	(<1)
Total	215	(100)	91	(100)	71	(100)	98	(100)	238	(100)	713	(100)

Figures in brackets are percentages (%).

Table 17 Incidence of non Enterobacteriaceae isolated from positive test kits in relation to laboratory, source and kit

Source	1.1	1.2	1.3	1.4	1.8	2.1	2.2	2.3	3.1
Medium									
Colilert	0	0	2	3	0	11	2	3	1
MMGM	0	1	2	1	0	7	2	1	0
PAB	1	0	0	1	0	0	0	0	1
Colitrace	1	0	0	0	0	17	0	1	4
LMX	1	0	1	1	1	16	0	4	1
Total	3	1	5	6	1	51	4	9	7

Source	3.6	3.7	4.1	4.2
Medium				
Colilert	0	0	0	2
MMGM	1	0	0	4
PAB	0	0	0	5
Colitrace	3	1	1	6
LMX	0	0	1	7
Total	4	1	2	24

Source	5.11	5.6	6.3	7.1	8.2	8.3	Total
Medium							
Colilert	1	0	0	0	1	0	26
MMGM	0	2	0	1	0	0	22
PAB	0	5	1	0	0	0	14
Colitrace	4	1	1	0	0	4	44
LMX	2	0	1	0	0	2	38
Total	7	8	3	1	1	6	144

See Appendix B for laboratory and source identification, for example source 4.2 refers to laboratory 4—Source 2. See also Table 7.

Appendix A Detailed Results of Phase 2 Comparisons of PA Test Kits

Table A1 Comparison of MF and MPN

E. coli		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	7	22	9	22	16	44
Membrane	+ve	24	47	17	52	41	99

Ent. cloacae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	5	19	6	25	11	44
Membrane	+ve	24	52	15	54	39	106

K. pneumoniae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	18	29	13	32	31	61
Membrane	+ve	22	31	24	31	46	62

Table A2 Comparison of MF and PAB

E. coli		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	12	17	15	16	27	33
Membrane	+ve	27	44	26	43	53	87

Ent. cloacae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	7	17	11	20	18	37
Membrane	+ve	14	62	14	55	28	117

K. pneumoniae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	22	25	19	26	41	51
Membrane	+ve	29	24	25	30	54	54

Comparison of MPN and PAB

E. coli		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	14	17	9	17	23	34
MPN	+ve	25	44	32	42	57	86

Ent. cloacae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	8	21	6	15	14	36
MPN	+ve	13	8	19	60	32	118

K. pneumoniae		Laboratory 4 MPN		Laboratory 5 MPN		Total MPN	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	22	18	16	21	38	39
MPN	+ve	29	31	28	35	57	66

Table A3 Comparison of MF and LMX

E. coli		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	15	14	17	14	32	28
MF	+ve	40	31	37	32	77	63

Ent. cloacae		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	13	11	2	29	15	40
MF	+ve	13	63	24	45	37	108

K. pneumoniae		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	14	33	17	28	31	61
MF	+ve	26	27	26	29	52	56

Comparison of MPN and LMX

E. coli		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	16	15	13	13	29	28
MPN	+ve	39	30	41	33	80	63

Ent. cloacae		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	4	25	4	17	8	42
MPN	+ve	22	49	22	57	44	106

K. pneumoniae		Laboratory 4 LMX		Laboratory 5 LMX		Total LMX	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	17	23	14	23	31	46
MPN	+ve	23	37	29	34	52	71

Table A4 Comparison of MF and Colitrace

E. coli		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	14	15	8	23	22	38
MF	+ve	15	56	17	52	32	108

Ent. cloacae		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	7	17	6	25	13	42
MF	+ve	21	55	18	51	39	106

K. pneumoniae		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	14	33	11	34	25	67
MF	+ve	25	28	18	37	43	65

Comparison of MPN and Colitrace

E. coli		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	9	22	5	21	14	43
MPN	+ve	20	49	20	54	40	103

Ent. cloacae		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	8	21	3	18	11	39
MPN	+ve	20	51	21	58	41	109

K. pneumoniae		Laboratory 4 Colitrace		Laboratory 5 Colitrace		Total Colitrace	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	15	25	10	27	25	52
MPN	+ve	24	36	19	44	43	80

Table A5 Comparison of MF and Colilert

E. coli		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	16	13	20	11	36	24
MF	+ve	34	37	35	34	69	71

Ent. cloacae		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	9	15	7	24	16	39
MF	+ve	14	62	14	55	28	117

K. pneumoniae		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	18	29	17	28	35	57
MF	+ve	14	39	29	26	43	65

Comparison of MPN and Colilert

E. coli		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	16	15	13	13	29	28
MPN	+ve	34	35	42	32	76	67

Ent. cloacae		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	7	22	6	15	13	37
MPN	+ve	16	55	15	64	31	119

K. pneumoniae		Laboratory 4 Colilert		Laboratory 5 Colilert		Total Colilert	
		-ve	+ve	-ve	+ve	-ve	+ve
	-ve	15	25	15	22	30	47
MPN	+ve	17	43	31	32	48	75

Appendix B Phase 3 Summary of results processed by individual laboratories

Laboratory 1

230 samples were examined between Dec '93 and Mar '94 and they originated from 10 sources.

Source	Number of samples	Type
1	45	lake
2	45	lake
3	35	lake
4	55	well
5	15	stream
6	5	stream
7	3	SW
8	17	SUW
9	7	SW
10	3	PWS

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	230	116	72	101	39	120	108	155 (73)	137 (82)	135 (57)
1	45	31	20	27	11	34	28	40 (20)	33 (23)	32 (13)
2	45	23	13	19	6	20	21	35 (14)	28 (14)	31 (13)
3	35	13	8	10	5	17	14	18 (9)	18 (10)	22 (8)
4	55	15	10	11	4	17	14	27 (8)	21 (7)	20 (4)
5	15	15	11	15	5	15	15	15 (14)	15 (13)	15 (12)
6	5	5	3	5	2	5	5	4 (3)	3 (3)	4 (3)
7	3	0	0	0	0	0	0	0 (0)	0 (0)	0 (0)
8	17	11	6	11	6	9	6	12 (4)	16 (12)	7 (4)
9	7	0	0	0	0	0	2	1 (0)	0 (0)	1 (0)
10	3	3	1	3	0	3	3	3 (1)	3 (0)	3 (0)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli*.
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 2

202 samples were examined between Dec '93 and Mar '94 and they originated from 3 sources:

Source	Number of samples	Type
1	129	SW
2	22	PWS
3	51	PWS

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	202	98	21	63	2	60	20	158 (4)	63 (3)	149 (4)
1	129	62	11	41	0	38	7	119 (2)	45 (1)	116 (1)
2	22	13	5	9	1	11	7	14 (2)	5 (2)	16 (2)
3	51	23	5	13	1	11	6	25 (0)	13 (0)	17 (1)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 3

204 samples were examined between Nov '93 and Mar '94 and they originated from 23 sources, but 17 of these were different sites at the same recreational water. No obvious differences in comparative results between methods were apparent and so these samples have been grouped and appear as source 1.

Source	Number of samples	Type
1	137	SRW
2	2	PWS
3	2	PWS
4	24	PWS
5	10	PWS
6	10	PWS
7	19	PWS

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	204	159	140	153	48	169	133	183 (54)	153 (54)	177 (55)
1	137	120	107	117	30	120	100	133 (36)	111 (35)	134 (37)
2	2	2	2	2	1	2	2	2 (2)	2 (2)	2 (2)
3	24	2	2	2	2	2	2	2 (2)	2 (2)	2 (2)
4	10	6	6	6	3	15	8	12 (4)	10 (3)	10 (2)
5	10	6	4	5	3	8	4	7 (3)	5 (3)	6 (3)
6	10	8	7	8	1	8	4	10 (2)	9 (2)	9 (1)
7	19	15	12	13	8	14	13	17 (5)	14 (7)	14 (8)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 4

200 samples were examined between Nov '93 and Mar '94 and they originated from 4 sources:-

Source	Number of samples	Type
1	80	PWS
2	90	PWS
3	5	PWS
4	25	PWS

		POSITIVES											
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX		Colitrace		Colilert	
ALL	200	138	114	124	96	150	178	181	(110)	146	(110)	137	(101)
1	80	41	37	38	31	53	59	62	(42)	44	(33)	44	(33)
2	90	72	52	61	40	71	89	89	(42)	74	(53)	67	(43)
3	5	5	5	5	5	5	5	5	(5)	5	(5)	5	(5)
4	25	20	20	20	20	21	25	25	(21)	23	(19)	21	(20)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 5

219 samples were examined between Nov '93 and Feb '94 and they originated from 11 sources.

Source	Number of samples	Type
1	10	PWS
2	20	PWS
3	10	PWS
4	20	PWS
5	19	PWS
6	59	PWS
7	2	PWS
8	37	PWS
9	7	PWS
10	10	PWS
11	28	PWS

		POSITIVES									
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX		Colitrace		Colilert	
ALL	219	81	66	80	56	117	104	155	(46)	108	(47)	148	(32)
1	10	2	2	2	0	1	0	0	(0)	0	(0)	1	(0)
2	20	16	14	16	14	17	16	19	(15)	19	(8)	20	(10)
3	10	2	2	2	0	2	1	3	(1)	2	(0)	3	(0)
4	20	9	9	9	8	10	10	13	(9)	12	(0)	14	(0)
5	19	16	10	15	6	16	19	19	(8)	16	(12)	19	(11)
6	59	11	10	11	10	38	27	51	(5)	28	(16)	39	(5)
7	2	0	0	0	0	0	0	0	(0)	0	(0)	0	(0)
8	37	0	0	0	0	4	0	8	(0)	2	(0)	10	(0)
9	7	0	0	0	0	1	3	7	(0)	1	(0)	7	(0)
10	7	0	0	0	0	0	1	7	(0)	0	(0)	7	(0)
11	28	25	19	25	18	28	27	28	(8)	28	(11)	28	(6)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 6

123 samples were examined between Oct '93 and Mar '94 and they originated from numerous PWS but, because of practical problems and unexpected results, many sources were used on too few occasions to be analysed separately and have been combined as source code "88".

Source	Number of samples	Type
1	1	PWS
2	23	PWS
3	40	lake
4	1	lake
88	37	various PWS

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	123	79	72	78	32	55	54	83 (34)	61 (19)	79 (26)
1	12	0	0	0	0	0	0	0 (0)	0 (0)	0 (0)
2	23	11	10	10	1	4	3	7 (0)	9 (1)	8 (2)
3	40	29	26	29	23	26	25	34 (17)	29 (4)	31 (9)
4	11	9	6	9	3	6	4	10 (0)	4 (1)	11 (1)
88	37	30	30	30	5	19	22	32 (17)	19 (13)	28 (14)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 7

198 samples were examined between Nov '93 and Feb '94 and they originated from three sources:

Source	Number of samples	Type
1	135	PWS
2	35	hospital supply
3	28	storage tank

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	198	107	50	90	18	87	25	101 (19)	81 (17)	97 (11)
1	135	103	46	86	18	87	25	101 (19)	81 (17)	97 (11)
2	35	0	0	0	0	0	0	0 (0)	0 (0)	0 (0)
3	28	4	4	4	0	0	0	0 (0)	0 (0)	0 (0)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Laboratory 8

33 samples were examined between Dec '93 and Feb '94 and they originated from 9 sources, but there were only 1 or 2 samples from 7 sources so these results have been combined as source "1":

Source	Number of samples	Type
1	10	SW and PWS
2	4	reservoir
3	19	reservoir

		POSITIVES								
Source	No. of Samples	MF	¶	§	#	MMGM	PAB	LMX	Colitrace	Colilert
ALL	33	19	15	17	11	15	15	21 (11)	17 (3)	13 (1)
1	10	5	3	5	1	4	4	7 (3)	5 (3)	6 (0)
2	4	2	2	2	2	4	2	4 (0)	3 (2)	3 (0)
3	19	10	10	10	8	7	9	10 (8)	9 (8)	4 (1)

¶ = confirmation of at least one colony as defined by the old definition.
§ = confirmation of at least one colony as defined by the old or new definition.
# = confirmation of at least one colony as *E.coli.*
Figures in brackets indicate results showing colour and fluorescence.

Appendix C Results from SCA trial

Results from previously unpublished SCA trial where a total of 156 samples were examined by two methods using MF and Colilert. The trial was undertaken in 1990 and carried out in four laboratories. Three laboratories tested mostly treated waters and one laboratory tested mostly raw waters.

Paired results for coliform organism counts

		Colilert	
		negative	positive
MF	negative	65	8
	positive	27	56

Paired results for *E. coli* counts

		Colilert	
		negative	positive
MF	negative	111	5
	positive	10	30

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