EVALUATION OF RAPID METHODS FOR THE DETECTION OF INDICATOR ORGANISMS IN DRINKING WATER

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Background and motivation

Detection of indicator bacteria are of primary importance in the assessment of the microbiological quality of drinking water. In particular, coliforms and *E.coli* are used as indicators of faecal pollution in water and as criterion of operational parameters in the water supply industry. Two basic procedures are used for the enumeration and detection of indicator bacteria from drinking water. These procedures include the multiple tube fermentation method (MTF) which provides a most-probable-number (MPN) analysis after growth of coliforms in liquid medium. The membrane filter (MF) technique enumerates total coliforms on the surface of agar by providing a colony forming unit per 100ml count (SABS Standard Methods 221-1990; APHA, AWWA, and WPCF, 1992). Methods should yield results rapidly to allow for speedy remedial action during water pollution events. Presently, the duration to obtain a negative result (a water sample containing no coliform bacteria) is 24h. A complete analysis for total coliforms and faecal coliforms, which requires confirmation procedures, can require 72h for a final result (SABS Standard Methods 221-1990).

Aim

The aim of the project was to evaluate the available new methodologies for the enumeration of bacterial indicators and confirmation of *E. coli* to establish whether more rapid and practical methods exist which may be used as an alternative to the conventional MF methods, described in the SABS Standard Methods.

Methodology

The Total Coliform Rule, promulgated by the U.S. Environmental Protection Agency on 31 December 1990, is based on the presence-absence test and is now the mandatory test for the examination of drinking water. New media based on the Defined Substrate Methodology (DST) have been developed for direct and simultaneous detection of coliforms and *E.coli* without confirmation. DST utilizes two indicator substrates, *o*-nitro-phenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG), which are combined to simultaneously detect total coliforms and *E.coli*. Total coliforms produce the enzyme β-galactosidase, which hydrolyses ONPG and thereby releases *o*-nitrophenol, which produces a yellow colour. *E.coli* produces the enzyme β-glucuronidase, which hydrolyses MUG to form a fluorescent compound.

Colilert and Colisure, which incorporate both MUG and ONPG, and can be used in a MPN or a presence absence (P/A) format to assess the bacteriological quality of drinking water, were therefore compared with the MF method for the detection of coliform bacteria and *E.coli*.

In the USA, the EPA and the American Water Works Association have established a system for the evaluation of alternate test procedures for rapid and nationwide adoption of new and minor revisions of analytical technology. This study was conducted based on these accepted procedures for the comparison of two or more methodologies for the detection of coliform bacteria and the confirmation of *E.coli* in drinking water.

To obtain a variety of isolates representative of both target and non-target isolates, the study was done using primary sewage effluent. Organisms were stressed by exposure to chlorination similar to those in drinking water treatment facilities. To determine the specificity of the proposed tests, the false positive- and false negative error were calculated.

Results

The results showed that the CL P/A method was equivalent to the reference method (MF) for the detection of total coliforms and *E.coli*. Simultaneous detection of coliform bacteria and *E.coli* within 24h, without having to perform additional confirmatory tests, was possible using the Colilert method. CS yielded unsatisfactory results which compared poorly to the MF method. Statistical analysis of the data showed no significant difference between the CL and reference method in detecting total coliforms and *E.coli* in water samples. Statistical analysis demonstrated that the CS method was significantly less efficient than the reference method (MF) in detecting both total coliforms and *E.coli*.

Conclusions and Recommendation

From the results of this study it can be recommended that a DST method such as Colilert be included as an acceptable method for the identification of both total coliforms and confirmation of *E.coli*.

There is a generally recognized need for methods that permit rapid estimation of the bacteriological quality of water. Further studies examining the suitability of available rapid detection methods that require less than 24h to obtain results, are therefore recommended.

A further consideration which should be taken into account when choosing a P/A test for routine use is the cost per test. A cost evaluation revealed that the commercially available kits were more expensive than the conventional testing methods for coliforms and *E.coli*. Although costs for the confirmation of coliform bacteria and *E.coli* using the conventional methods are minimal the major cost contribution, however, lies in the increased labour and the time it requires. As no confirmatory tests are necessary when using CL and CS, the major cost saving lies in decreased time and labour.

Technology transfer

The SABS has indicated that the results of this study could also be taken in account during the revision of methods for the detection of indicator bacteria in drinking water planned for the end of 1995.

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1. INTRODUCTION

The total coliform group of bacteria is the principal indicator used to evaluate the microbial quality of drinking water. The coliform group is considered a reliable indicator of potential of faecal pollution in drinking water. Detection and enumeration of indicator bacteria are therefore of primary importance in the microbiological quality control of water. In particular, coliforms and *E.coli* are used by many in the water supply industry as a criterion of operational parameters and indicators for faecal pollution. Included in the SABS specifications and other South African guidelines for assessing the quality of drinking water are total and faecal coliforms with confirmation of *E.coli*.

There are two methods commonly applied for the enumeration of these indicator bacteria from drinking water. The multiple tube fermentation (MTF) provides a most-probable-number (MPN) analysis after growth of coliforms in liquid medium. The membrane filter (MF) technique enumerates total coliforms on the surface of agar by providing a colony forming unit per 100mℓ count (SABS Standard Methods 221-1990; APHA, AWWA, and WPCF, 1992). The principles described in the SABS Standard Methods 221-1990 for enumeration of coliform and faecal coliform bacteria are similar to the International Standards Organisations (ISO) methods. These methods depend on lactose fermentation to detect the presence of coliforms. The MPN and MF methods have both been studied extensively and are approved for regulatory monitoring purposes (LeChevallier, *et al.*, 1983; Covert, 1985; Standard Methods, 1992; SABS 221-1990). A complete analysis for coliforms with confirmation of *E.coli* requires 72 hours for a result according to the SABS Standard Method.

The MPN and MF methods both have several inherent properties of which the most restricting is the time required to obtain confirmed results for coliform and faecal coliform bacteria present in a water sample. This is mainly as a result of the inability to differentiate faecal coliforms from total coliforms without the performance of either separate confirmatory tests for faecal coliform bacteria or identification of *E. coli*, and the subjective nature of interpretation of the analytical methods.

The Total Coliform Rule, promulgated by the U.S. Environmental Protection Agency (EPA) on 31 December 1990, changed the manner of reporting total coliforms from numbers per 100mℓ of sample to a presence-absence (P/A) form of reporting percentages of samples that are positive. This prompted the re-examination of traditional methods. New media have been developed for direct and simultaneous detection of coliforms and *E.coli* without confirmation. The new methodology is a refinement of defined substrate technology (DST) applied in clinical laboratories. DST is unique because it directs the metabolism of the target bacteria to specific indicator nutrients. The formula for the new media is in a stable powder form that can be added directly to the sample or vice versa. In the new methodology, two active substrates, *o*-nitro-phenyl-β-D-galactopyranoside(ONPG)and4-methylumbelliferyl-β-D-glucuronide(MUG), are combined to simultaneously detect total coliforms and *E.coli* (Olson, *et al.*, 1991). Total coliforms produce the enzyme β-galactosidase, which hydrolyses ONPG and thereby releases *o*-nitrophenol, which produces a yellow colour. *E.coli* produces the enzyme β-glucuronidase, which hydrolyses MUG to form a fluorescent compound.

The efficiency of the DST has been evaluated through studies directed by the US-EPA, (Covert, *et al.*, 1989; Edberg, *et al.*, 1988 and 1989), and water utilities (McCarty, *et al.*, 1990). These studies indicated the methodology to be effective for the detection of coliform bacteria and *E.coli* in untreated source water and treated drinking water. Coliform bacteria, subjected to injurious physicochemical conditions of drinking water, although exhibiting a longer lag

phase before reaching log phase, can also be detected successfully (McFeter, *et al.*, 1993; Edberg, *et al.*, 1988).

To establish whether more rapid and practical methods exist which may be used as an alternative to the conventional MF methods described in the SABS Standard Methods, (hereafter referred to as the reference method), the new methodologies (hereafter referred to as the proposed methods), were compared and evaluated with the reference method for the detection of coliform bacteria and *E.coli*. The proposed tests used for this investigation were Colilert and Colisure which incorporate both MUG and ONPG and can be used in a MPN or a presence absence (P/A) format. Colilert and Colisure were chosen for this study as these were the only commercially kits available at the time.

2. PROJECT OBJECTIVES

The aim of this study was to examine the comparability of Colilert (CL) and Colisure (CS) systems with the SABS MF method to detect low numbers of total coliform bacteria and *E.coli* subjected to chlorine stress .

With regard to the new methods special attention was given to the following aspects:

i) enumeration of 1 total coliform per 100 mℓ in a maximum of 24h
ii) simultaneous, specific enumeration of one *E.coli* per 100mℓ in the same test
iii) requirements for confirmatory tests
iv) ability to detect injured coliforms
v) sample inoculation method
vi) interpretation of results
vii) costs per test

3. MATERIALS AND METHODS

In the USA, the EPA and the American Water Works Association have established a system for the evaluation of alternate test procedures for rapid nationwide adoption of new and minor revisions of analytical technology. This study was conducted based on these accepted procedures for the comparison of two or more methodologies for the detection of coliform bacteria and the confirmation of *E.coli* in drinking water.

3.1 Samples

Non-chlorinated primary effluent containing no industrial effluent was collected in sterile polypropylene bottles at five different geographical locations (Gauteng, Western Cape, Kwazulu/Natal and two from Northern Transvaal). Non-chlorinated primary sewage effluent was preferred because it is a good source of total and faecal coliforms as well as a wide range of *E.coli* strains. To reduce chlorine demand 100mℓ of each of the original samples were sonicated for 10min (to prevent clumping) and made up to one litre in oxidant-free Milli Q^{50} sterilised water. Microorganisms in the samples were thereafter stressed by exposure to chlorine to reduce viability 1-3 logs. Chlorine was neutralised with 0.8mℓ of a 10% (w/v) sodium thiosulphate solution/ℓ. Samples were further diluted with oxidant-free sterilised water (Milli Q^{50}) to achieve a target concentration of 3-10 viable coliforms per 100mℓ.

3.2 Test protocol

Sufficient water sample from each geographical area was prepared to perform a simultaneous split sample analysis by the CL, CS and MF techniques. For each water sample the following analyses were performed: a 10-tube MPN assay (using 10 mℓ/tube) for Colilert and Colisure and a 100mℓ analyses on both m-FC and m-Endo agar LES. Prepared samples were repeatedly and thoroughly hand shaken during the experiment. For each of the 5 samples a total of 10 repeats were performed. Therefore, for each sample there were 100 tubes for Colilert and 100 tubes for Colisure and 10 membrane filtrations on each of the agars (m-Endo agar LES and m-FC agar).

3.3 Controls

Pseudomonas aeruginosa (CCRC 10944), *Klebsiella pneumoniae* (CCRC 10692) and *E.coli* (DSM 1576) cultures were used as comparative positive and negative control bacteria for the reference method and both CL and CS.

In addition, an *Aeromonas hydrophila* culture, originating from an environmental water sample, identified using the API 20E system, was tested over a range of concentrations (0-10^7 organisms per mℓ). The dilutions were prepared, inoculated and incubated at 35±0.5° C for 24h and 28h. The response of both CL and CS was recorded.

3.4 Coliform MF

Total coliforms were enumerated by the MF method with m-Endo agar LES as prescribed in SABS Standard Methods 221-1990. Plates were incubated at 35±0.5° C and read at 24h. Colonies exhibiting the green metallic shine were picked and confirmed for gas production in lactose peptone water. A culture that produced gas within 24 to 48 h was considered positive for coliforms (SABS 221-1990).

3.5 Faecal coliform MF and confirmation of *E.coli*

Faecal coliforms were enumerated by the MF method with m-FC agar. Plates were incubated at 44.5±0.25° C and read at 24h. Suspect colonies exhibiting a blue centre with a translucent periphery were picked and confirmed with lactose peptone water. A culture that produced gas within 48h was considered positive for faecal coliforms.To confirm the presence of *E.coli* confirmed cultures of faecal coliforms were subcultured from the lactose peptone water to tryptone water and incubated at 44.5±0.25° C for 24h. After incubation 0,3-0,5 mℓ Kovacs reagent were added to each tube to test for the formation of indole. The development of a red colour denotes the presence of indole and confirms the presence of *E.coli* (SABS 221-1990).

3.6 P-A tests

Autoanalysis Colilert (CL) manufactured by Environetics, Inc., and Colisure (CS) manufactured by the Millipore Corporation, USA, were used to examine the comparability of these methods with the SABS methods.

The Colilert and Colisure test reagents were in powder form in plastic pillows and screw-cap glass tubes, respectively. Each reagent was added to a 100mℓ sample, which was shaken to dissolve the powder. The sample mixture was pipetted in 10mℓ aliquots into 10 sterile glass tubes to quantify by means of the MPN method. Capped tubes were incubated for 24-28h at

35±0.5°C. To determine the concentration of total coliforms and *E.coli* per 100mℓ, the number of positive tubes per sample was compared to the standard MPN probability tables.

CL tubes exhibiting a yellow colour and CS tubes exhibiting a dark red/purple (magenta) colour were considered confirmed total coliforms. Each coliform positive tube was checked for fluorescence using a long wavelength ultraviolet lamp (366 nm). If fluorescence (yellow/green for CL and bright blue for CS) was exhibited the presence of *E.coli* was considered confirmed.

3.7 Substrate specificity of Colilert and Colisure

Evaluations for coliform bacteria and *E.coli* were conducted using CL and CS methods as described by the manufacturers. From each of the 5 samples sufficient replicate analyses were performed to afford 10 positive and 10 negative responses (tubes), for the target response organisms, coliforms and *E.coli*. A false positive error was recorded if a non-target organism produced the reaction expected from the target organism. An undetected target error or false negative was recorded if the target organism failed to produce the expected positive reaction in the test procedure.

3.8 Evaluation of the false positive error

Procedures followed for the evaluation of false positive error were according to the requirements prescribed by the US-EPA (Covert, 1985) with a modification for confirmation of faecal coliforms and *E.coli*. The SABS procedures were followed instead of the USA Standard Method (AWWA, APHA and WPCF, 1992) prescribed by the US-EPA; *i.e.* brilliant-green bile lactose broth (BGLB) and production of gas for coliform detection at 35 ± 0.5°C and faecal coliform detection at 44.5 ± 0.25°C followed by the determination of the production of indole in tryptone water for the confirmation of *E.coli*.

3.8.1 Procedure

The procedure is schematically presented in Figure 1.

For each of 50 coliform positive tubes and each of 50 *E. coli* positive tubes the following tests were performed:

- A loopful from each positive tube was streaked out for single colonies on m-Endo agar LES and incubated at 35 ± 0.5°C.
- From the isolated lactose-positive colonies one colony representative of each type of morphology was streaked out for single colonies on nutrient agar and incubated at 35 ± 0.5°C.
- A loopful of each morphological different colony on nutrient agar was transferred to a separate tube of lauryl tryptose broth (LTB).
- Tubes inoculated with presumptive coliforms were incubated at 35 ± 0.5°C. Tubes with presumptive faecal coliforms or *E. coli* were incubated at 44.5 ± 0.25°C. for 48h.
- Presumptive coliform and faecal coliform bacteria (LTB tubes with gas production) were confirmed by transferring 1mℓ of the LTB broth to BGLB broth.
- Tubes were incubated at 35 ± 0.5°C and 44.5 ± 0.25°C for 48h.
- Gas production confirmed the presence of coliforms (35 ± 0.5°C).
- Gas production at 44.5 ± 0.25°C were considered confirmed faecal coliforms.
- To confirm the presence of *E.coli*, confirmed cultures of faecal coliforms were

subcultured from the LTB to tryptone water and incubated at 44.5 ± 0.25° C for 24h.
- After incubation 0,3-0,5 mℓ Kovacs reagent were added to each tube to test for the formation of indole.
- The development of a red colour denotes the presence of indole and confirms the presence of *E.coli*.

Calculation

False positive error =A/B

Where A= Number of proposed method (new method) positive response units in which the target organism was not verified by the reference tests

and B = Total number of proposed method positive response units.

3.9 Evaluation of the undetected target error.

3.9.1 Procedure

The procedure is schematically presented in Figure 2.

For each of the 50 tubes negative for coliforms and each of the 50 tubes negative for *E. coli* the following test were performed:

- One mℓ of broth from each negative CL and CS tube was transferred to LTB broth and incubated at 35 ± 0.5° C for 24-48h.
- Tubes with gas were streaked for single colonies on nutrient agar and incubated at 35 ± 0.5° C for 24h.
- Each morphologically different type of colony on the nutrient agar was prepared for a gram stain and oxidase test.
- Gram negative non-sporing, oxidase negative colonies were transferred to LTB and incubated at 35 ± 0.5° C for 24-48h.
- Presumptive coliform bacteria (tubes with gas production) were confirmed by transferring 1mℓ to BGLB tubes and were incubated at 35 ± 0.5° C for 24-48h.
- Production of gas confirmed the presence of coliform bacteria.
- The confirmation of *E.coli* was achieved by inoculating tryptone water tubes with each morphologically different colony isolated on the nutrient agar and incubation at 44 ± 0.25° C for 24h.
- After incubation 0,3 - 0,5 mℓ Kovacs reagent was added to each tube to test for the formation of indole.
- The development of a red colour denotes the presence of indole and confirms the presence of *E.coli* (SABS 221-1990).

Each response unit where the target organism was undetected by the proposed method (new method), but was observed to be positive by the reference medium was reverified by using the proposed method.

Calculation

Undetected target error = C/(C+D)

Where C = Number of proposed method undetected target response units in which the target organism was positive by the reference tests but was reverified as undetected by the proposed medium.

and D = Number of proposed method positive response units in which the target organism was positive by the reference tests.

3.10 Statistics

The positive and negative P/A responses from the CL and CS tests were compared against the responses of the MF test.

The Cochran-Mantel-Haenszel test analyzes a set of 2x2 contingency tables by combining individual table results into a single test statistic. The contingency tables were developed by using the number of positive and negative tubes by each method as the columns and two methods as rows. The proportions of positive tubes from the MF, CL and CS tests were compared by determining whether the detection rate of positive tubes was the same for both methods by the chi-square statistic. The hypothesis tested was that there is no difference in detection rates by the two methods.

Precision of the two proposed methods was examined by comparing the variability among the number of positive tubes from the ten replicate analyses. The variance was calculated for each method and sample and then compared by the Wilcoxon signed ranks test. This test uses the sign and magnitude of the rank of the differences between the pairs of sample variances to determine statistical significance. The t-test was also performed to compare the two proposed tests with the reference method.

All statistical tests were performed at the alpha = 0.05 level of significance.

FIGURE 1: Schematic Outline of Completed Tests for Determining the False Positive Error for Coliforms and *E.coli*

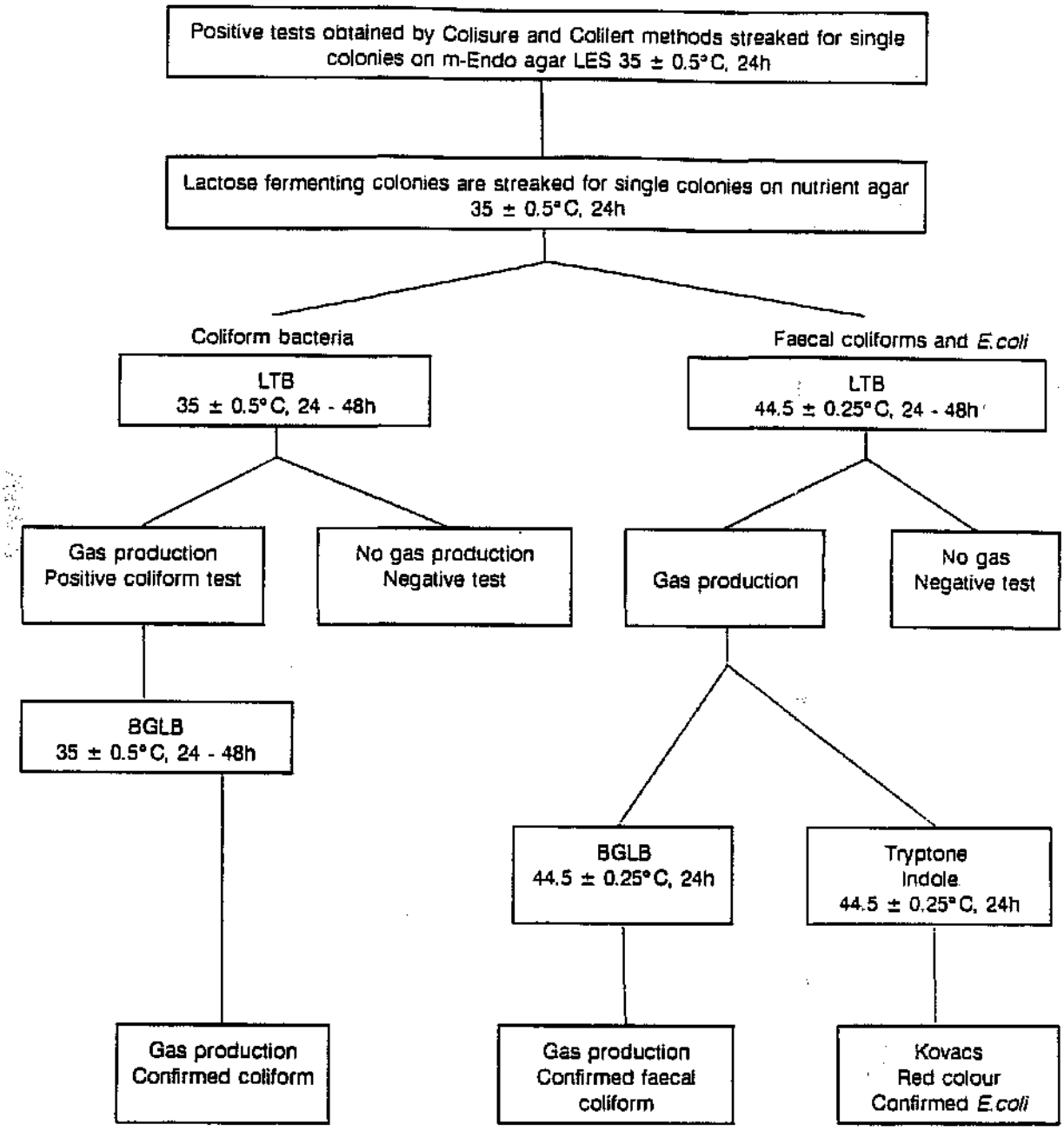
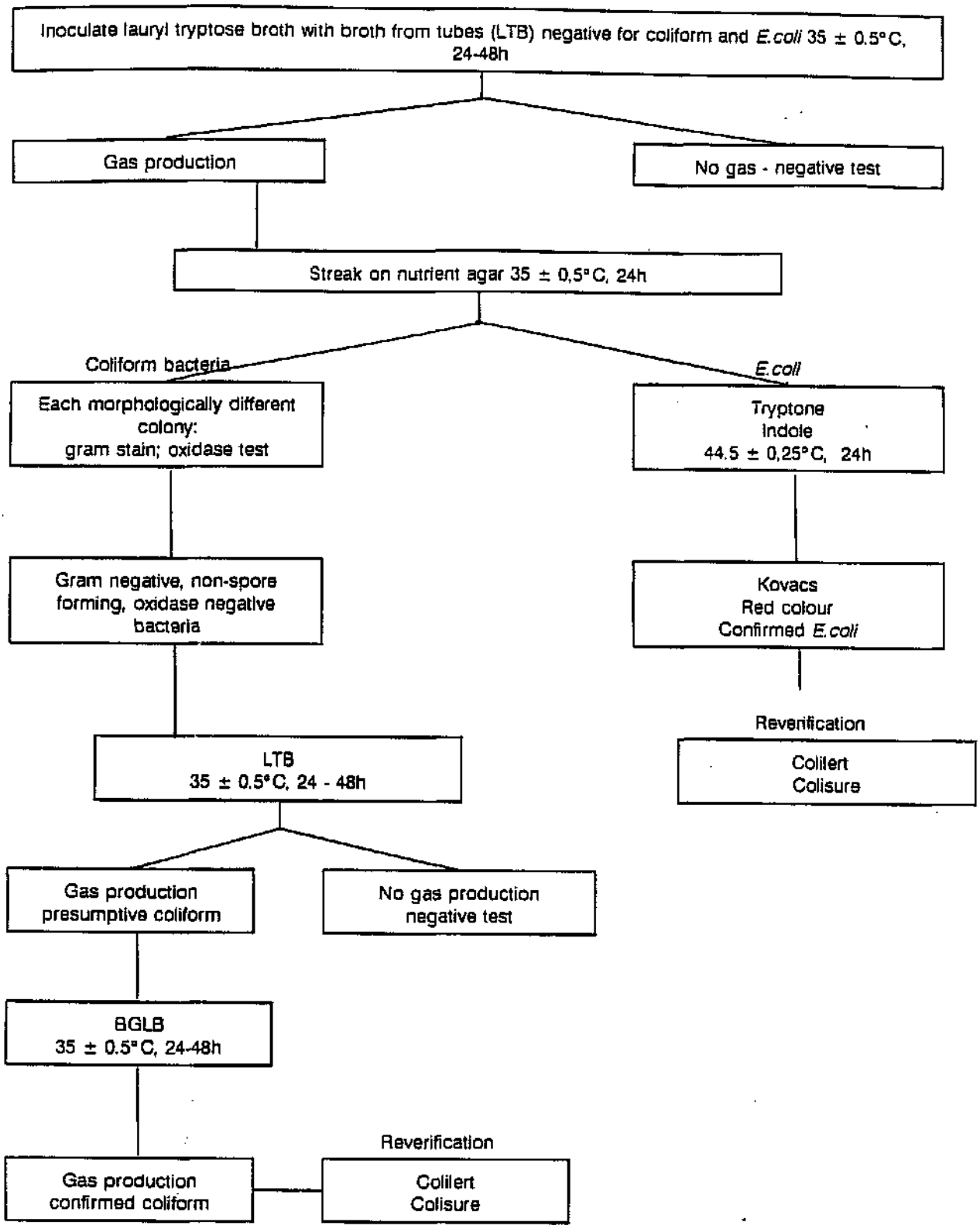


FIGURE 2: Schematic Outline of Completed Tests for Determining the Undetected Target Error for Coliforms and *E.coli*



4. RESULTS

4.1 Comparison of MF, CL and CS.

Results for the total number of positive observations obtained for the comparative evaluation of 50 tests for both the commercial P/A tests and the reference method (MF) are shown in Tables 1 and 2. The data in Table 1 indicate only a slight difference in the total numbers of positive tests observed for CL (50 positive observations) and MF techniques (48 positive observations) for the detection of total coliforms in the water samples. When results obtained for the MF technique and CS were compared it was indicated that the MF technique showed a higher level of recovery for total coliform bacteria. CS showed positive results for 41 of the 50 tests, whereas 48 of the 50 tests were positive when using the MF technique.

Comparing the MF method and CL test for the detection of *E.coli*, showed that the CL test detected a higher number of *E.coli* positive water samples than both the MF and CS methods (Table 2). The MF method detected *E.coli* in 80% of the samples and the CL test detected *E.coli* in 90% of the samples (Table 3). Results obtained for the comparison of CS with the MF technique indicated that the MF method was superior for the detection of *E.coli*, showing positive observations for 80% of the samples. The CS method detected *E.coli* in only 46% of the samples tested (Table 2).

Agreement (both positive and negative) between the reference and two test methods is presented in Tables 4 and 5. Agreement between the CL and MF method occurred on 48 (48+0) and 40 (39+1) of the 50 analyses for total coliforms and *E.coli* respectively (Tables 4 and 5). Agreement between the CS and MF method occurred on 41 (40+1) and only 24 (20+4) of the 50 analyses for total coliforms and *E.coli* respectively (Tables 4 and 5).

Percentage false positive and false negative errors as described in 3.8.1 and 3.9.1 are presented in Tables 6 and 7 respectively. The total percentage error is presented in Table 8. CS produced more false positive results than CL. CL tests resulted in 7.4% and 7.2% false positive coliform and *E.coli* tests, respectively, while CS produced an unexpectedly high 29% false positive *E.coli* results (Table 7). CL produced 6.6% more false negative *E.coli* results than CS (Table 6). The CL test resulted in 19.7% false negative and positive errors for *E.coli*, whereas the CS test accounted for 34.9% (Table 8). Therefore the total percentage error rate for CS was 27.8% greater than that of the CL test for the detection of *E.coli*.

Aeromonas hydrophila, at densities of zero to 20 organisms/mℓ induced a positive result in CL after a 24h incubation period. CS was less sensitive at this bacterial concentration and only showed positive results after a further 4h incubation period. Densities above 20 organisms /mℓ showed positive results for CL and CS after 24h of incubation. Fluorescence, indicating a false positive detection of *E.coli*, never occurred in any of these tests.

4.2 Statistical analysis

Statistical analysis of the data (Table 9) showed no significant difference between the CL and reference method in detecting total coliforms and *E.coli* in water samples. Statistical analysis demonstrated that the CS method was significantly less efficient than the reference (MF) in detecting both total coliforms and *E.coli*.

Table 1 : Number of positive results obtained for the reference method (MF), Colilert and Colisure for the detection of coliform bacteria

Sample	No. Tests	Reference method	Proposed methods	
		Total coliform No. positive	No. positive by Colilert	No. positive by Colisure
1	10	8	10	9
2	10	10	10	10
3	10	10	10	5
4	10	10	10	7
5	10	10	10	10

Table 2 : Number of positive results obtained for the reference method (MF), Colilert and Colisure for the detection of *E.coli*

Sample	No. Tests	Reference method	Proposed methods	
		E.coli No. positive	No. positive by Colilert	No. positive by Colisure
1	10	5	9	4
2	10	10	10	3
3	10	10	8	4
4	10	10	8	4
5	10	5	10	8

Table 3 : % Total Number of Samples Positive using the MF Method, Colilert and Colisure for the Detection of Coliform Bacteria and *E. coli*

No. tests	Reference method		Proposed methods			
	m-Endo agar LES	m-FC agar	Colilert		Colisure	
	Coliform	*E. coli*	Coliform	*E.coli*	Coliform	*E.coli*
50	96	80	100	90	82	46

Table 4 : Comparison of total coliform detection for the reference test versus the Colilert and Colisure tests

Number of samples				
Ref. method for total coliforms	Colilert		Colisure	
	positive	negative	positive	negative
positive	48	0	40	8
negative	2	0	1	1

Table 5 : Comparison of *E.coli* detection for the reference test versus the Colilert and Colisure tests

Number of samples				
Ref. method for *E.coli*	CL		CS	
	positive	negative	positive	negative
positive	39	4	20	23
negative	6	1	3	4

Table 6 : % False Positive Error Observed using Colilert and Colisure

Coliform bacteria		*E.coli*	
Colilert	Colisure	Colilert	Colisure
7.4	8.4	7.2	29

Table 7 : % Negative Target Error Observed Using Colilert and Colisure

Coliform bacteria		*E.coli*	
Colilert	Colisure	Colilert	Colisure
ND*	6.3	12.5	5.9

* ND = Not detected as no negative results were obtained for the Colilert method

Table 8 : Comparison of Total % Error Between False Positives and Negatives in the Colilert and Colisure Tests

	Coliform bacteria		Total	*E.coli*		Total
	Colilert	Colisure		Colilert	Colisure	
False positive	7.4	8.4	15.8	7.2	29	36.2
False negative	ND*	6.3	6.3	12.5	5.9	18.4
Total	7.4	14.7	22.1	19.7	34.9	54.6

* ND = Not detected

Table 9 : Statistical analyses of comparisons of Colilert and Colisure with MF method based on presence or absence for the detection of total coliforms and *E.coli*

Statistical method / Comparison of reference method with:	Cochran-Mantel-Haenszel test	Wilcoxon signed ranks test	One-sided t-test
Total coliforms Colilert	0.528 sig.lev=0.467 CL=ref	Z=0.89 p=0.37 CL=ref	t=1.17 sig.lev=0.122 CL=ref
Total coliforms Colisure	3.86 sig.lev=0.049 ref>CS	Z=2.01 p=0.044 ref>CS	t=2.27 sig.lev=0.013 ref>CS
E.coli Colilert	#2.96 sig.lev=0.083 CL=ref	Z=0.51 p=0.61 CL=ref	t=-0.61 sig.lev=0.729 CL=ref
E.coli Colisure	10.833 sig.lev=0.001 ref>CS	Z=3.42 p<0.0000 ref>CS	t=4.61 sig.lev=0.000 ref>CS

\# Two additional test statistics were calculated according to similar associations, showing the CL method to be equivalent or superior to the reference method.

5. DISCUSSION

This study was undertaken to examine the comparability of Colilert (CL) and Colisure (CS) systems with the SABS MF method to detect low numbers of total coliform bacteria and *E.coli* subjected to chlorine stress to mimic conditions experienced in treated drinking water.

CL and CS have previously been compared with MF, MPN and P/A procedures from *Standard Methods* and found to produce equivalent and even superior data for the detection of coliform bacteria and *E.coli* (Katamay, 1990; Edberg, *et al.*, 1988 and 1989; McFeters, *et al.*, 1994). Results obtained from this study showed that CL is as effective for detecting coliform bacteria and *E.coli* as the MF methods prescribed by the SABS Standard Method 221-1990. However, results obtained for the CS test showed an unacceptable difference in results for both coliforms and *E.coli*.

Contradictory results have been reported for a number of studies in which the specificity and sensitivity of commercial P/A tests for the detection of coliform bacteria and *E.coli* were investigated and compared to the MPN and MF methods. Hall and Moyer (1989) and Covert, *et al.*, (1989) indicated that a statistically significant difference existed between the MPN test and the CL system for the detection of coliforms. These findings showed that the MPN test was superior for coliform detection. MF procedures used for the detection of total coliforms and faecal coliforms, generally revealed good agreement among the P/A tests. However in a study comparing CL and Coliquik P/A tests with the MF technique using m-FC agar, Clark, *et al.*, (1991) found poor agreement between the MF and the CL and Coliquik tests for *E. coli* detection in treated water samples. A statistically significant difference was shown between both CL and Coliquik and the MF method for the detection of *E.coli*. No significant difference was, however, shown between CL, Coliquik and the MF method for the detection of *E. coli* from untreated surface water. Disagreement between CL and Coliquik methods was also reported by Olson, *et al.*, (1991). Coliquik was not included in this comparison because it could no longer be obtained at the time of the study. McFeters, *et al.*, (1993), showed in a comparative study of CS with the MF method for the detection of *E.coli* that CS yielded superior results for the detection of both coliforms and *E. coli*. A possible explanation for the inconsistency of the results obtained for P/A methods for this study and differences reported in literature may be due to differences in sample types (McCarty, *et al.*, 1992). Clark, *et al.*, (1991) used predominantly drinking water samples. Researchers have used source water, cisterns, well water diluted with dechlorinated tap water, river water and diluted primary effluent chlorinated to obtain stressed bacteria. This study followed the established system developed in collaboration with the US-EPA and American Water Works Association where test samples contain naturally occurring target organisms.

Concern has been expressed regarding the number of false-negative results obtained for these studies. Olson, *et al.*,(1991) attributed the lack of consistency among P/A tests primarily to the high number of false negative results produced. In this study false negative *E.coli* results were slightly elevated for CL, but contrary to findings in literature, data obtained for this study produced exceptionally high levels of false positive *E.coli* results for the CS test. Elevated false positive results for the CS method concurred with difficulties experienced in assessing results because colour formation was abnormally slow. The 24h incubation period was generally not sufficient for the expected colour formation of the CS formula. The additional 4h incubation period suggested by the manufacturers and investigations cited in literature was also not sufficient and complete colour formation was only observed after 48h. Data suggest that a proportion of the faecal and *E.coli* population could not utilise the ONPG or MUG upon initial inoculation. A possible explanation might be injury of bacteria due to chlorination, inability of the bacteria to quickly adapt to the substrate, substrate specificity, inability of substrate to enter the cell, or lack of expression of the gene or non-utilization of the MUG substrate by *E.coli* strains. (Olson, *et al.*, 1991; Katamay, 1990). Manufacturers specifically warn against incubation periods beyond 28h because the reagent system changes and heterotrophic bacteria may overcome the suppressant systems of the test after this time, yielding a false positive result. Covert, *et al.*, (1989) found that extension of the incubation time beyond the

48h period resulted in false positive ONPG CL tubes from *Aeromonas hydrophila* and *Pseudomonas spp.*

The occurrence of *Aeromonas hydrophila* in water supplies are important as it is a well known opportunistic pathogen and has been implicated as the causative agent of waterborne enteric infections. *Aeromonas hydrophila* have been shown to contain β-galactosidase but lack the permease to transport the substrate into the cell (Olson, *et al.*, 1991). It has, however, been suggested that *Aeromonas hydrophila* could produce possible interference when using CL and CS and other DST formulas. Edberg, *et al.*, (1988) found that ONPG-positive non-coliforms such as *Aeromonas hydrophila* do not yield positive CL test because the formula does not support their metabolism. They stated that detection of β-galactosidase containing organisms, therefore, will only occur at very high microbial concentrations (>20-100000/mℓ). In this study, on the other hand, it was found that CL and CS detected *Aeromonas hydrophila* at much lower densities. CL was more sensitive than CS and gave positive results after 24h of incubation when the bacterial density was 20 organisms /100mℓ water sample. An additional 4h incubation period was necessary for CS to produce a positive result for the same density of *Aeromonas hydrophila* bacteria. Bacterial densities above 20 organisms/mℓ induced positive colour changes for both CL and CS after 24h incubation. No false detection of *E.coli* was observed using *Aeromonas hydrophila* for both CL and CS.

Covert, *et al.*, (1989) and Edberg, *et al.*, (1988), suggested that the CL test should not be applied to source waters, effluents, or samples other than drinking water supplies unless the efficiency of the test with the particular water sample type has been established.

The most probable cause for the apparent insensitivity of the CS formula lies in the fact that this product has a storage temperature of 4°C. Delivery of the shipment from the USA to South Africa takes several days during which the product could have been exposed to a wide range of extreme temperatures.

A shelf life of at least 15 months with the requirement of a minimum-maximum storage temperature of 4-30°C makes CL a more attractive product than the CS test. It provides CL with the additional advantage of being used by small utilities and rural providers, especially during summer months. Once the formula is hydrated, the bacteria begin to grow, although more slowly at ambient than incubator temperature. No change in the bacterial composition of the water sample can take place after inoculation.

The observation that the concentration of bacteria in the inoculum largely determined the rapidity of colour formation for the CL and CS test during this study was in agreement with findings of other researchers (Katamay, 1990; Edberg, *et al.*, 1988). The reason for this is the manner in which the DST directs the metabolism of the target bacteria to the specific indicator nutrients. This direct metabolism occurs because only the target microbes can utilize the substrate. This also holds the advantage of inhibiting the growth of heterotrophic bacteria and minimizing interference of these organisms in the test.

Some of the performance characteristics of commercially available P/A tests which make them much more practical and efficient to perform than the current coliform detection methods are:

- Sensitivity to detect total coliforms and *E.coli* concentrations as low as 1CFU/100 mℓ
- Results in 24h, depending on coliform or *E.coli* concentration

- Simultaneous identification of coliforms and *E.coli*
- No confirmatory tests needed
- Specific *E.coli* identification to species with no additional work
- Configuration as either a P/A or MPN test
- Equal utilisation of small and large utilities
- Easy interpretation of results
- Easy inoculation of test
- Injured coliforms can be detected
- No equipment other than a 366 nm Ultraviolet lamp is necessary
- Allows small utilities to test as accurately as large ones
- Moderately trained individuals can interpret the results

6. COSTS ANALYSIS

Another consideration which should be taken into account when choosing a P/A test for routine use is the cost per test. An evaluation of the current costs in South Africa per test revealed the following:

Colilert:
R564.30 for 20 tests. R28.22 per test.
R4446.00 for 200 tests. R22.23 per test.

Colisure:
R926.82 for 20 tests. R46.34 per test.

MPN Colisure test:
R1575.48 for 20 tests. R78.78 per test.

Coliform test using m-Endo agar LES and three petri dishes.
R4.68 per test.

Faecal coliform/*E. coli* test using m-FC agar and three petri dishes.
R3.99 per test.

Costs for the confirmation of coliform bacteria and *E.coli* using the reference methods are not included because its monetary contribution per test is minimal. The major contribution in cost for confirmation tests lies in the increased labour and the time it requires.

Although these calculations show that it would be considerably more expensive to use the P/A formula instead of the MF method several other benefits in using the P/A tests should however be considered. Since there are no additional tests needed, an analysis will not extend through weekends which could result in a delay of 2-5 days in obtaining definitive results. Also, in contrast to other methods in which weekends added 2 more days to an analyses, it would be easy for an employee to test water on a Friday and have someone read the results on Saturday. The major cost saving associated with CL and CS therefore lies in decreased time and labour.

Capital Expenses

To determine the capital outlay for basic apparatus needed to start a membrane filtration facility in an existing laboratory versus using CL or CS kits, the following ranges of prices were obtained:

Equipment for Reference Method		Equipment for Proposed Methods	
MF	Costs	CL and CS	Cost
Membrane filters	R0.88-R3.20 / filter	Hand held ultraviolet light	R194.00-R915.00
Three filter holders	R366.00-R2487.00		
One 3-port manifold	R1493.00-R11245.00		
Vacuum pump	R1420.00-R2000.00		

Time requirement

The following table illustrates the time required, using the reference method versus the proposed method, to obtain a result for a negative sample and a positive sample.

Reference Method MF				Proposed Methods CL and CS			
Negative Result		Positive Result		Negative Result		Positive Result	
Coliform	*E.coli*	Coliform	*E.coli*	Coliform	*E.coli*	Coliform	*E.coli*
24h	24h	72h	72h	24h	24h	24h	24h

7. CONCLUSIONS AND RECOMMENDATIONS

The CL and CS tests can simultaneously detect coliform bacteria and *E.coli* from a water sample with no additional confirmatory tests. The CL test produced results within 24-28h. The CS test evaluated during this study was hampered by an inability of bacteria to grow sufficiently to evaluate colour formation within the expected 24-28h incubation period. The poor results obtained with CS may be due to problems encountered during transport of the reagents which highlights the importance of recommending only those methods not requiring refrigeration for storage. The tests are very simple to use and the colours produced by the total coliforms and the fluorescence generated by *E.coli* were distinct, and easy to read.

Although costs for testing water using the P/A methods are considerably higher than the costs using the MF technique a major cost saving associated with the P/A tests lies in the significantly shorter time required to perform the test.

The results showed that the CL P/A method was equivalent to the reference method (MF) for the detection of total coliforms and *E.coli*. CS yielded unsatisfactory results which compared poorly to the MF method. Statistical analysis of the data showed no significant difference between the CL and reference method in detecting total coliforms and *E.coli* in water samples. Statistical analysis demonstrated that the CS method was significantly less efficient than the reference method (MF) in detecting both total coliforms and *E.coli*.

From the results of this study it can be recommended that a DST method such as CL be included as an acceptable method for both the identification of total coliforms and confirmation of *E.coli*.

The SABS has indicated that the results of this study could also be taken in account during the revision of methods for the detection of indicator bacteria in drinking water planned for the end of 1995.

There is a generally recognized need for methods that permit rapid estimation of the bacteriological quality of water. Small utilities with only moderately trained personnel and developing communities will benefit from rapid and simple detection methods for assessing the bacteriological quality of potable water. Rapid, simple methods can be invaluable during emergencies involving water treatment plant failure, line breaks in a distribution network, or other disruptions to water supply caused by disasters. Further studies examining the suitability of available rapid detection methods that require less than 24h to obtain results, are therefore recommended.

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