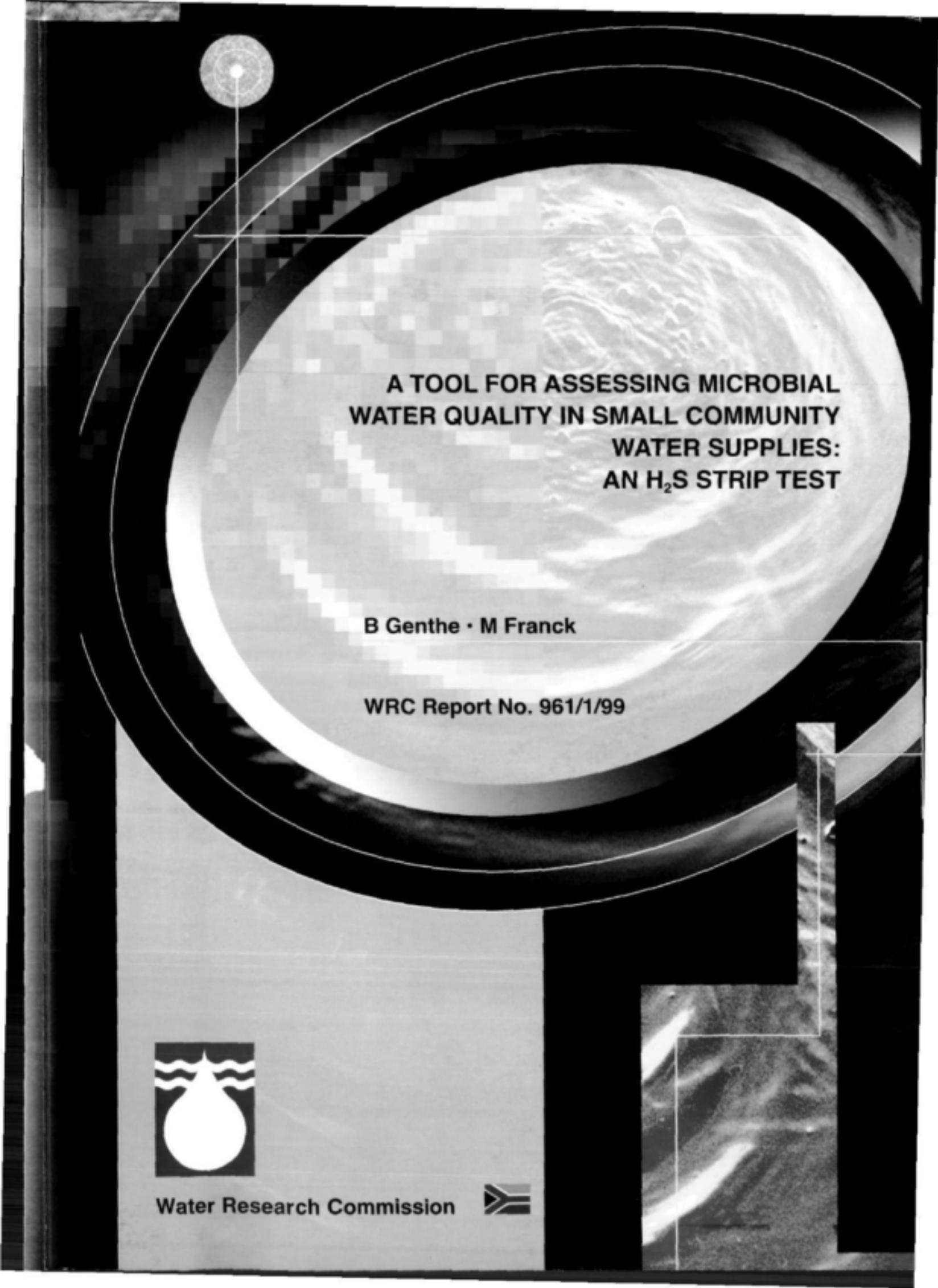

A TOOL FOR ASSESSING MICROBIAL WATER QUALITY IN SMALL COMMUNITY WATER SUPPLIES: AN H_2S STRIP TEST

B Genthe · M Franck

WRC Report No. 961/1/99

Water Research Commission

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B. Genthe and M. Franck

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EXECUTIVE SUMMARY:

Background and Motivation

Protection of public health relies on providing the public with safe and reliable drinking water. According to guidelines for drinking water, water intended for human consumption should be safe, which means that it should be free of pathogenic microorganisms. Water supplies in small communities in South Africa have been shown to have notoriously poor water quality with reports of 80% of samples tested failing drinking water quality guidelines.

This emphasises the need to have a rapid and reliable method which can be used by field workers, environmental health officers and community water committees to identify where potential problems exist in the drinking water quality management process.

A method has been described in the literature that has been successfully used in small communities for assessing water quality. This method is based on an H_2S strip test which turns the water sample black if it is contaminated. It therefore provides an easy and visual method for assessing water quality. The method has been tested for its relationship with coliforms and coliphages and a significant correlation between the H_2S strip test and the indicator organisms was found. The apparent benefit of this method is its ability to conduct the test at room temperature. This would allow water samples to be collected and assessment of the water quality could be carried out in the field without transporting it to a laboratory.

Aim

The project aims were:

- to test an H_2S strip method for its suitability as a water quality indicator
- to test the method for its suitability for detecting contamination of water supplies without requiring incubation at specific temperatures
- to test the H_2S strip method as a field kit for assessing the water quality of small community water supplies

Methodology

The method for the H_2S strip test was acquired and tested for its sensitivity, specificity and appropriateness as a water quality indicator. The method was compared to the traditional indicator organisms, namely heterotrophic bacteria, total coliforms and faecal coliforms. The sensitivity of the test was established in both the presence and absence of large concentrations of other organisms. The method was tested at both 35^{0}C and at room temperature.

Environmental water samples of varying water quality were tested using the H_2S strip test and 3 indicator organisms. A total of 415 samples were included in the comparison.

Results of the H_2S strip test are reported as positive or negative, whereas results of the indicator organisms are quantitative. Results of the indicator organisms therefore needed to be categorised as positive or negative for correlations to be calculated. Various levels of contamination were used to categorise the indicator organisms data. The results were also examined for total agreement *ie,* if both tests were positive, or both negative, this represents agreement, and if one was positive and the other negative, this represents disagreement.

Results

The sensitivity of the H_2S strip test proved capable of detecting as few as 2 organisms per sample in both the absence and presence of competing organisms.

Correlations between the H_2S strip test and indicator organisms were statistically significant, with faecal coliforms having the best correlation with the H_2S strip test after 48 hours incubation ($r = 0.80$), followed by total coliforms ($r = 0.4$), and poorer correlations being found with heterotrophic bacteria ($r = 0.3$). Percentage agreement was highest for faecal coliforms (on average > 80% agreement) with slightly higher agreement with incubation at room temperature compared to 35°C (86% vs 82%). If the test was conducted at room temperature the incubation time needed before reading the test was 48 hours. If the test is carried out at 35°C, 24 hours incubation is required.

Conclusions

The H_2S strip method is a sensitive test capable of detecting low levels of contamination in water samples. The statistically significant correlations with the traditional indicator organisms and high percentage agreement between the H_2S strip test and indicators illustrates its suitability as a water quality indicator.

The H_2S strip test was found to have higher correlations (r values) and higher percentage agreement with the indicator organisms when conducted at room temperature than at 35°C. A possible explanation for this is that the bacteria responsible for the H_2S production may have their optimum growth temperature at less than 35°C.

These results imply that it is a useful 'on -site' field test and is light, easy to use and portable. The raw materials do not require storage under refrigeration.

It is not suggested that the H_2S strip test be used as a replacement to the current water quality indicator organisms, but rather that it can be used in addition to those tests, particularly in areas where water quality testing would not have normally been carried out.

The use of this test will contribute towards the protection of public health, particularly in small communities, by allowing water quality to be assessed and adequately managed. Communities where water quality was previously not assessed on a regular and routine basis due to either logistical, skills or financial constraints will now be able to be routinely tested.

Recommendations for Future Research

Further research is required involving testing the method on field workers for its use in managing small water supply systems, and testing their attitudes regarding the applicability of the test. The method also needs to be tested under field conditions to determine the effect of factors such as varying and extreme temperatures; sunlight and incubation times. To be able to use the H_2S strip test in the proposed remote areas further research is required. Research will need to confirm that the test is suitable for these remote areas and that it has been adequately tested in the field to allow an endorsement for the use of this test to be made.

Technology Transfer

Preliminary tests are being carried out in the field with organisations involved in water quality management and analysis in rural communities. For instance, the test is currently being evaluated in the Northern Cape through interactions with PD Toens, in the Eastern Cape with Rural Support Services and in Gauteng/ Northern Province with Sego-Dolo Development. Each participant has agreed to provide feedback on the usefulness of the test and their experiences with it.

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The steering committee over the study period involved the following individuals:

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Dr M van der Walt	Water Research Commission
Mrs P Coubrough	Environmentek, CSIR
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Mr HAR Petrik	SABS
Mr N Lesufi	Dept. of Health
Dr A Kuhn	Dept. Water Affairs &Forestry

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- Des Visser and Ken de Witt of PD Toens and Associates
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- Claire Boffin of Rural Support Services
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1. INTRODUCTION

The transmission of disease via polluted water has been acknowledged as a source of concern with regards to public health for a long time. A wide range of illnesses, ranging from the acute, such as gastroenteritis, to the chronic, such as cancer, can be spread through contaminated drinking water. Protection of the public's health from water- related illnesses depends on the consumer having a safe and reliable drinking water. According to guidelines for drinking water, water intended for human consumption should be safe, palatable and aesthetically pleasing. This means that the water used for domestic purposes should be free of pathogenic microorganisms and other substances that may present a health risk.

How water is assessed to be 'safe' varies slightly according to guidelines and standards used throughout the world. It is impossible to test the water supplies for all potential pathogens related to waterborne disease for a number of reasons. These include:

- large numbers (100's) of potential pathogen that would need to be assessed
- the time involved to detect many pathogens could result in a delayed response in remedial action required if water is contaminated
- the costs of pathogen detection methods are in many cases extremely expensive
- detection methods do not exist for all potential waterborne pathogens

Indicator systems which are able to index the presence of pathogens are therefore used to ensure the safety of drinking water. As an indicator, an organism should fulfil a number of criteria:

- it should be present when a pathogen is present, and absent in unpolluted water
- it should be present in larger numbers than the potential pathogens
- it should survive as long as, and be as resistant to treatment as pathogens
- it should not be harmful to health
- it should be easy to identify

(Berg, 1978; DWAF, 1996, Genthe and Kfir, 1995; McNeill, 1985; WHO 1993)

Using these criteria, there is no indicator that meets the requirements as an ideal indicator. However, the traditional indicators used to assess drinking water quality include the coliform group and heterotrophic bacteria.

The indicator organism tests themselves have limitations or restrictions in that they require a trained technician to carry out the analyses, as well as requiring expensive laboratory materials and equipment, such as incubators, sterilization equipment, and filtration apparatus. In addition, water samples have to be analysed preferably within 6 hours of sampling, for results to be valid. Therefore, the monitoring of water supplies in remote areas is restricted by these requirements. Often no trained

personnel are available in the remote areas, equipped laboratories may be distant, and a lack of available funds makes it impossible to adequately monitor drinking water.

It has become increasingly obvious that a simple method needs to be developed or acquired that permits the evaluation of the sanitary quality of water for small community water supplies.

Small water supplies in South Africa have been shown to have notoriously poor water quality (Buthelezi *et al.*, 1997; CSIR, 1997) where 80% of small community water supplies were found to fail drinking water quality guidelines. This highlights the necessity to firstly have a rapid and reliable method which can be used by field workers, environmental health officers and community water committees to pinpoint where potential problems exist, and secondly, where further investigations or remedial action is necessary (by DWA&F for example). In addition, a simple, visual method to assess water quality could be used to increase the awareness of the communities themselves to potential health risks associated with contaminated water.

A method has been described that has been successfully used in small communities in South America, Indonesia and India, for assessing water quality (Castillo *et al*, 1994; Martins, *et al*, 1996; Kromoredjo and Fujioka 1991; Venkobachar *et al* , 1994). This method is based on an H_2S strip test which causes contaminated water to turn black, and hence provides a very effective visual mechanism for illustrating contamination of water supplies. This method was tested in the field in South American villages and found to be very effective at increasing the awareness of the communities regarding water quality issues. The method was tested for its relationship with coliforms and coliphages and a significant correlation between the H_2S strip test, coliforms, and coliphages was observed.

This H_2S strip test provides a potentially effective method for use in South Africa for increasing the awareness of environmental health officers and communities of water quality issues as well as providing a screening method for assessing water quality.

The benefits of the H_2S test comprise its good correlation with traditional water quality indicators; good correlation between H_2S production and presence of Salmonella species (particularly in the absence of coliforms)(Gawthorne *et al* , 1996); its portability allowing field tests to be carried out; relative low costs; and its ease of use allowing trained but unqualified staff to perform the test.

The H_2S method was developed by Manja *et al* (1982) as an on-site test and is based on the detection of hydrogen sulphide. Alternative on-site and simple tests include the "defined substrate technology" tests and include kits such as Colilert (Environetics, Inc.) and Colisure (Millipore Corporation). An evaluation of these methods compared to the traditional indicator organisms is described in a previous WRC report (Genthe and du Preez, 1995) The disadvantages of these methods are that they require laboratory facilities (UV lamps and incubators) and are more

expensive than the traditional test methods.

Several researches throughout the world have used the H_2S test and found it to be successful at detecting contamination of drinking water (Castillo *et al*, 1994; Venkobachar *et al*, 1994; Gawthorne *et al* ,1996; Martins *et al*, 1996, Pillai *et al*, 1998) The H_2S method is a very simple one, requiring the incubation of the water sample in a sterile bottle containing the reagents which allow detection of H_2S production. The bottles are incubated and the development of a black colour indicates a positive reaction.

One apparent benefit investigated in this study is the reported ability to use the test at room temperature (Pillai *et al.*, 1998). (This would have particular benefit in the South African context in that it would allow water supplies in the remote rural areas to be assessed. As the test may be carried out at room temperature it would allow water samples to be collected and the assessment could begin immediately without transporting it to the nearest laboratory. In addition, the tests could be carried out by trained staff, but not necessarily qualified microbiologists. For instance Environmental Health Officers would be able to obtain preliminary information on the water quality of drinking water supplies under their jurisdiction. The test would not replace the tradition drinking water quality assessments as recommended by the Departments of Water Affairs & Forestry (DWAF) and Health (DoH). It could however be used as an early warning or screening of water supplies to indicate whether immediate follow-up investigations are necessary.

PROJECT AIMS:

The aims of the project were:

- to test an H_2S strip method for its suitability as a water quality indicator
- to test the method for its suitability for detecting contamination of water supplies without requiring incubation at 37^0C.
- to test the H_2S strip method as a field kit for assessing the water quality of small community water supplies

2. METHODS AND MATERIALS

2.1 Preparation of H_2S test medium

The original description of the H_2S test medium (Manja (1982) incorporating the modified (improved) method described by Venkobachar et al (1994) was prepared as follows:

- 40g peptone
- 3g K_2HPO_4
- 1.5g ferric ammonium citrate
- 2g sodium thiosulphate
- 2ml teepol
- 25mg L-cysteine
- 100ml distilled water

2.2 Preparation of H_2S strip test sample bottles

This H_2S medium (1ml aliquotes) was used to impregnated strips of folded paper towel of approximately 50cm^2 (5X10cm tightly rolled paper towel). The folded strips of paper containing the solution were sterilised and placed in pre-sterilised 40 ml plastic sample bottles.

2.3 H_2S strip test method

Prepared sample bottles were filled with the water sample being analysed to a pre-measured 20ml mark. Samples were then incubated at either 37^{0}C or room temperature (22-25^{0}C) and examined for H_2S production after 18 hours (overnight) followed by 12 hour intervals over a period of 72 hours. The formation of a black colouration in the sample bottles was recorded as a positive H_2S result. [Initially 44^{0}C was also included as an incubation temperature, but after the first 50 water samples were analysed it was decided to discontinue this incubation temperature as the results were rarely positive.]

2.4 Experimental procedure

A total of 415 samples were included in the assessment of the H_2S strip test. Various types of water samples, ranging from uncontaminated drinking water samples to contaminated environmental samples were collected and tested using the H_2S strip test. General descriptions of the types of water tested in the study are provided in Table A1, Appendix 1. Water samples were also tested for the traditional indicator organisms, namely, heterotrophic bacteria, total coliforms and faecal coliforms as described in SABS-221 (1991) (*ie*, the pour plate and membrane filtration methods, respectively).

2.5 Specificity test

As a quality control of the H_2S strip test, both positive and negative controls were carried out. *Citrobacter freundii* and *Proteus vulgaris* were used as positive controls, and *Escherichia coli* and *Salmonella typhi* were used as negative controls. (*Salmonella typhi* is not an H_2S producer whereas other *Salmonella species* are H_2S positive, for example *Salmonella typhimurium*.)

2.6 Sensitivity of H_2S test

Dilutions of known amounts of an H_2S producer (*Proteus vulgaris*) were made and tested with the H_2S strip test to establish the minimum number of organisms that the strip test was capable of detecting.

2.6.1. Sensitivity of H_2S test in presence of H_2S negative organisms

In addition, serial dilutions of known concentrations of both a positive control (*Proteus vulgaris*) and other organisms found in contaminated water samples (but negative H_2S production, namely, *Enterobacter, Klebsiella*, and *E coli* at a concentration of 10^6 organisms) were tested to establish whether non-H_2S producers would cause inhibition or reduce the sensitivity of the test when using it with contaminated environmental samples.

2.7 Analysis of results: correlation analyses

As the H_2S strip test is based on a positive / negative result, the H_2S strip test was compared to the 3 indicator organisms using correlation analysis (providing r values and significance or p values) of categorical data. The indicator organism results were classified according to whether the guidelines were complied to. For instance, level 1 uses the recommended drinking water quality guideline values as a cut-off value to be classified as positive for the indicator organism tests. Analyses using this level would be considered positive if heterotrophic bacteria exceed 100/ml, total coliforms of 5/100ml or faecal coliform counts >0/100ml. If the concentrations are less than this then the result would be classified as negative. Using level 2 as a cut-off range for positive or negative classification, any results for heterotrophic bacteria less than 1000/ml would be considered negative and any total and faecal coliform count less than 10/100ml would also be considered as a negative result. If level 3 was used for classification of the water, any sample with more than 10 000 heterotrophic bacteria /ml or 100/100ml total or faecal coliforms, would be considered to be positive and waters with less than these counts would be considered to be negative. Using level 4 as a cut-off value, water would be classified as positive if more than 100 000 heterotrophic bacteria /ml or 1 000 total or faecal coliforms /100ml were present. If less than these numbers were present the water would be classified as negative using level 4 as a classification criterion.

Correlation between H_2Sstrip test results and indicator organism levels were tested using these various cut-off levels to categorise data according to concentrations of organisms as described above and in Table 2.1.

Table 2.1: Levels of bacterial counts used to define data categorically (*ie*, as a +)

microbial parameter	level 1 exceeds guidelines	level 2 low level contamination	level 3 medium level contamination	level 4 high level contamination
heterotrophic bacteria	>100/ml	>1 000/ml	>10 000/ml	>100 000/ml
total coliforms	>5/100ml	>10/100ml	>100/100ml	>1 000/100ml
faecal coliforms	>0/100ml	>10/100ml	>100/100ml	>1 000/100ml

3. RESULTS

3.1 Specificity and Sensitivity Analysis:

Results of the positive and negative controls (specificity) are provided in Table 3.1 below, and results for the sensitivity tests are presented in Tables 3.2 and 3.3. Table 3.2 represents the sensitivity of the H_2S test in the absence of other potentially competing organisms. The test was able to detect approximately 2 to 4 organisms in the 20ml sample. Results were available within overnight incubation for all concentrations of organisms tested. In the presence of other non-H_2S producing organisms (at a concentration of 10^6/ 20ml sample) the sensitivity was **not** reduced, as the test was still able to detect approximately 2 organisms in the 20ml sample. However, when lower numbers of H_2S producing organisms were present, the results were available after a longer incubation period, with 48 hours required when less than 10 H_2S organisms were present.

This discrepancy between the time that results can be read (Tables 3.2 and 3.3) according to the concentration of organisms present (both H_2S producers and non-H_2S producers) unfortunately does not allow for potential quantification of the H_2S test using time as a criterion. It is impossible to know whether a test sample contains only H_2S producing bacteria or other non-H_2S producers as well.

Table 3.1 Positive and negative controls for H_2S test (specificity)

Positive control		Negative controls	
Proteus vulgaris	+	*Salmonella typhi*	-
Citrobacter freundi	+	*Escherichia coli*	-

Table 3.2 Sensitivity of H_2S test

Number of cells (*Proteus vulgaris*)	positive or negative H_2S	time for response	Number of cells (*Proteus vulgaris*)	positive or negative H_2S	time for response
10^6	+++	O/N*	10^2	+++	O/N
10^5	+++	O/N	10^1	+++	O/N
10^4	+++	O/N	<10	+++	O/N
10^3	+++	O/N	2-4	+++	O/N

* O/N = overnight

Table 3.3 Sensitivity of H_2S test in presence of a mixture of non-H_2S producers (*Enterobacter, Klebsiella* and *E coli*) at a concentration of 10^6 organisms per test

Number of cells (*Proteus vulgaris*)	positive or negative H_2S	time for response (h)	Number of cells (*Proteus vulgaris*)	positive or negative H_2S	time for response
10^6	+++	O/N*	10^2	+++	36 h
10^5	+++	O/N	10^1	+++	48 h
10^4	+++	O/N	<10	+++	48 h
10^3	+++	24 h	±2	+++	48 h

* O/N = overnight

Table 3.4 and Figure 3.1 provide a representation of the number of positive results of the total 415 samples tested, after 18h to 72h incubation(at 12h intervals) at the 2 temperatures tested (22°C and 35°C). At 22°C the majority of samples became positive after 48h incubation, whereas at 35°C a large number of the samples that were positive could be read by 24h incubation.

Table 3.4 Summary statistics : Number of samples H_2S positive after various hours incubation

T°C	Hours incubation (h) before reading H_2S strip test					
22 °C	18h	24h	36h	48h	60h	72h
no. samples +	13	106	216	231	245	248
35 °C	18h	24h	36h	48h	60h	72h
no. samples +	203	237	254	257	264	264

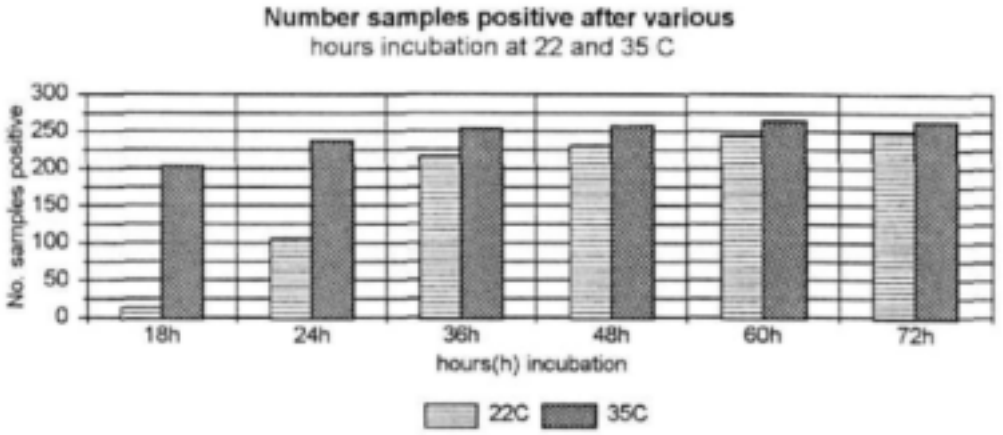


Figure 3.1 Summary statistics: Number of samples H_2S positive after various incubation times

Table 3.5 Summary statistics: Number samples classified as positive or negative according to various cut-off concentrations of indicator organisms (levels1-4)

HPC>100 (level 1)	Total coliforms > 5 (level 1)	Faecal coliforms > 0 (level 1)	HPC >1000 (level 2)	Total coliforms >10 (level 2)	Faecal coliforms >10 (level 2)
221	153	225	163	129	174
HPC> 10^4 (level 3)	Total coliforms >100 (level 3)	Faecal coliforms >100 (level 3)	HPC >10^5 (level 4)	Total coliforms >1000 (level 4)	Faecal coliforms >1000 (level 4)
88	89	138	46	38	24

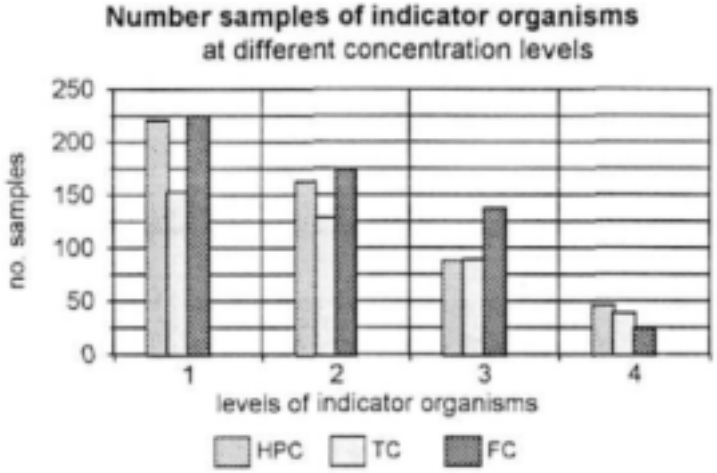


Figure 3.2 Summary statistics: Number of samples with indicator organisms at various concentration levels

Table 3.5 and Figure 3.2 provide summary information relating the number of samples classified as positive or negative (*ie*, categorical classification) according to the various levels of concentrations of indicator organisms as described in section 2.7. It can be seen that the samples analysed in the H_2S strip test had a large range on concentration levels. Approximately half of the samples analysed contained concentrations above the drinking water quality guidelines (level 1). A low percentage were in the range of high level contamination *(ie*, level 4 with HPC $>10^5$ /ml'; total and faecal coliforms >1000/100ml).

Figures 3.3 -3.6 illustrate the *positive agreement* between the H_2S strip test and the indicator organisms using the 4 different cut-off levels to classify the indicator organism response as positive or negative. Positive agreement refers to the number of samples that were positive for **both** H_2S strip test and indicator organism. In general, the faecal coliforms have the highest numbers of positive agreement when the data is categorised or classified at level 2 or 3 (Figures 3.4&3.5). Positive agreement between heterotrophic bacteria and H_2S strip is also high at the level 1 classification of data (Figure 3.3).

The analysis of agreement is presented in more detail in Tables 3.6-3.11 illustrating both positive and negative agreement between the H_2S strip test and the 3 different indicator organisms at both 22°C and 35°C.

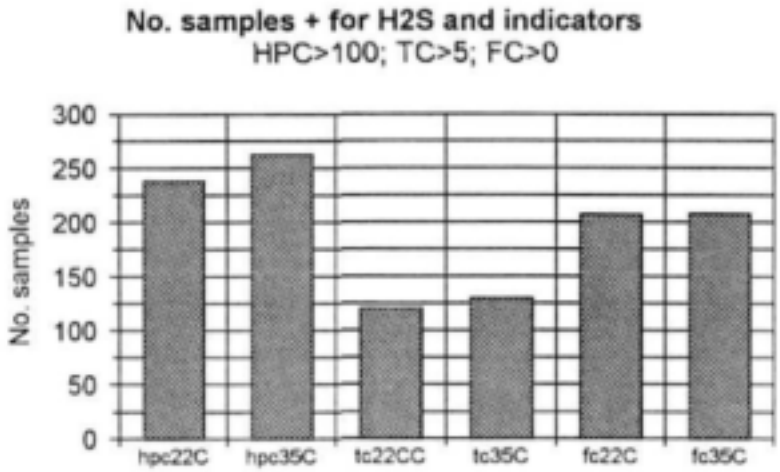


Figure 3.3 Percentage positive agreement at level 1
hpc = heterotrophic bacteria; tc = total coliforms and
fc = faecal coliforms

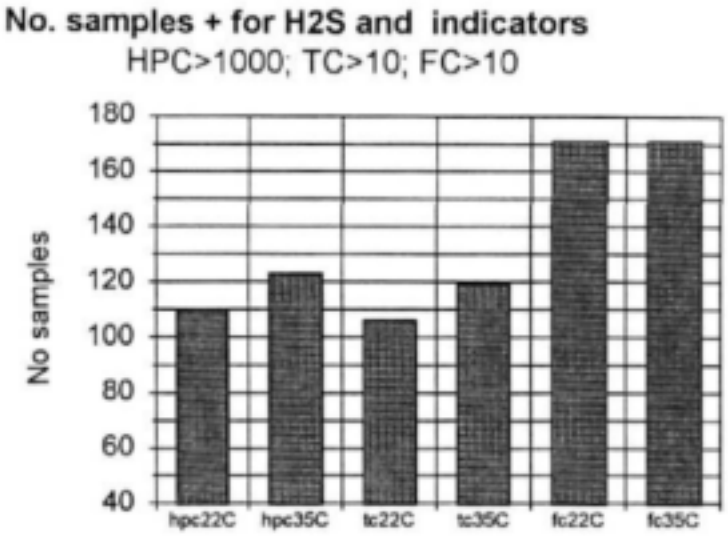


Figure 3.4 Percentage positive agreement at level 2
hpc = heterotrophic bacteria; tc = total coliforms and
fc = faecal coliforms

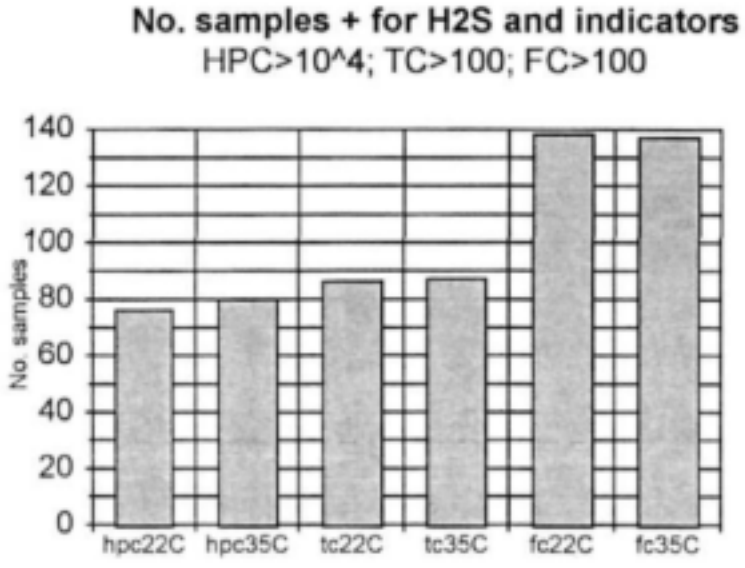


Figure 3.5 Percentage positive agreement at level 3
hpc = heterotrophic bacteria; tc = total coliforms and
fc = faecal coliforms

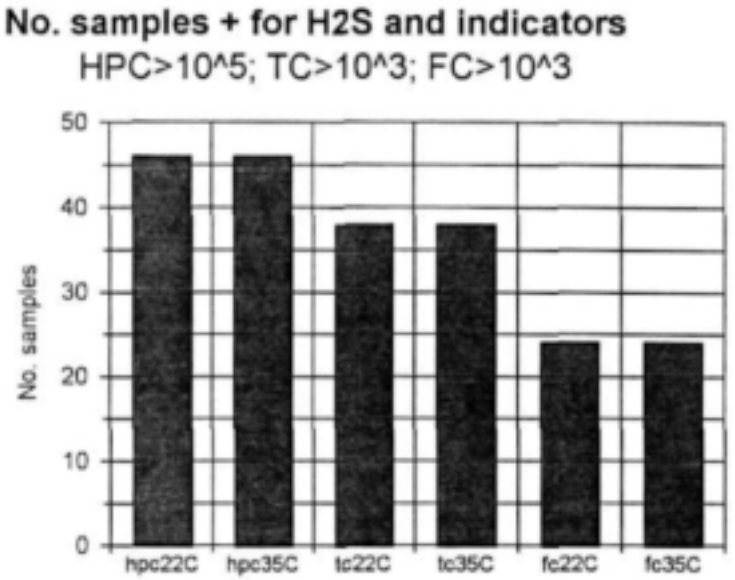


Figure 3.6 Percentage positive agreement at level 4
hpc = heterotrophic bacteria; tc = total coliforms; and
fc = faecal coliforms

Total agreement is highest for faecal coliforms (Tables 3.10 & 3.11) with over 350 of the 415 (84%) samples having agreement. The best agreement is found when the data for the faecal coliform counts is classified according to level 1 - drinking water quality guideline levels. At 22°C, 356 of the 415 samples (86%) showed total agreement, in comparison to 342 (82%) at 35°C (Tables 3.10 & 3.11). Lack of agreement where the H_2S strip test was negative when the indicator organism test was positive (the last column in Tables 3.6-3.11) occurred far less frequently than the reverse situation. In other words, the H_2S strip test did not fail to detect contamination as often as the individual indicator tests failed to detect contamination. This was particularly evident where contamination levels were detected using heterotrophic bacteria.

The lack of agreement in the categories using high levels of contamination to classify the data could be due to the nature of the classification system. For instance, in level 4 for total and faecal coliform counts, if counts are less than 100/100ml they would be classified as negative. The H_2S strip test would be positive at levels of contamination as high as this, and hence the lower levels of agreement.

Table 3.6: Percentage agreement: Heterotrophic bacteria (HPC) and H_2S strip test at 22°C

Heterotrophic plate count 22°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ HPC - H_2S	-HPC +H_2S
level 1[1]	145	91	236(57%)	76	103
level 2	110	114	224(54%)	53	138
level 3	76	155	231(56%)	12	172
level 4	46	167	213(51%)	0	202

Table 3.7: Percentage agreement: Heterotrophic bacteria (HPC) and H_2S strip test at 35°C

Heterotrophic plate count 35°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ HPC - H_2S	-HPC +H_2S
level 1[1]	155	85	240(58%)	65	108
level 2	123	110	233(56%)	40	140
level 3	80	142	222(54%)	8	183
level 4	46	150	196(47%)	0	217

Table 3.8: Percentage agreement: Total coliform (TC) count and H_2S strip test at 22°C

Total Coliforms 22°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ TC - H_2S	-TC +H_2S
level 1[1]	120	134	254(61%)	33	128
level 2	106	144	250(60%)	23	142
level 3	86	164	250(60%)	3	162
level 4	38	167	205(49%)	0	210

[1] levels described in text

[1] Levels described in text

Table 3.9: Percentage agreement: Total coliform (TC) count and H_2S strip test at 35°C

Total coliforms 35°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ TC - H_2S	-TC + H_2S
level 1[1]	130	128	258(62%)	22	133
level 2	119	141	260(63%)	9	144
level 3	87	149	236(57%)	1	176
level 4	38	150	188(45%)	0	225

[1] levels described in text

Table 3.10: Percentage agreement: Faecal coliform (FC) count and H_2S strip test at 22°C

Faecal coliforms 22°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ FC - H_2S	-FC + H_2S
level 1[1]	207	149	356(86%)	18	41
level 2	171	164	335(81%)	3	77
level 3	138	167	305(74%)	0	110
level 4	24	167	193(47%)	0	224

[1] levels described in text

Table 3.11: Percentage agreement: Faecal coliform (FC) count and H_2S strip test at 35°C

Faecal coliforms 35°C

Levels of indicator organisms	Agreement ++	Agreement --	Total Agreement	+ FC - H_2S	-FC + H_2S
level 1[1]	208	134	342(82%)	16	55
level 2	171	148	319(77%)	2	92
level 3	137	149	286(69%)	1	126
level 4	24	150	174(42%)	0	239

[1] levels described in text

Correlations between the 3 indicator organisms and the H_2S strip test after 18 - 72 hours incubation (at 12 hour intervals) at both 22°Cand 35°C are provided in Figures 3.7 and 3.8. The individual r values cannot be clearly distinguished in these figures, but what is immediately apparent is that the correlation for faecal coliforms at levels 1, 2 & 3 are higher than for the other 2 indicator organisms tested. Details of correlation (r) values together with the significance levels (p values) are provided in Appendix 1 Tables A2-A5. For easy visual comparison, Figure 3.9 provides a summary representation of correlations between the H_2S strip test and the three indicator organisms when the data was categorised using level 1 as a classification system. In addition, Figures 3.10-3.15 illustrate the correlations between the H_2S strip test and individual indicator organisms at the 4 different levels for a single temperature.

At 22°C, correlations between the H_2S strip test and heterotrophic bacteria are highest after the H_2S strip test has been incubated for 24 hours before reading (Figure 3.10) whereas, at 35°C, the correlations are highest after 18 hours incubation (Figure 3.11) if high levels of contamination are used as the classification system (levels 3 & 4). If lower levels are used, then the highest correlations are found after 24 hours incubation of the H_2S strip test.

For total coliforms, 24 hours incubation also provides the highest correlation values at 22°C at all classification levels (Figure 3.12). At 35°C, the highest correlations are found for levels 1 and 2 after 48 hours incubation and after 24 hours incubation if classified at levels 3 and 4 (Figure 3.13).

Correlations for faecal coliforms are better with the H_2S strip test being used at 22°C after 48 hours incubation compared to incubation at 35°C (Figures 3.4 & 3.15, and Appendix 1,Tables A2-A5). This is confirmed in the tables of percentage agreement (Tables 3.9 & 3.10) where total agreement is also higher when the H_2S strip test is used at room temperature 22°C.

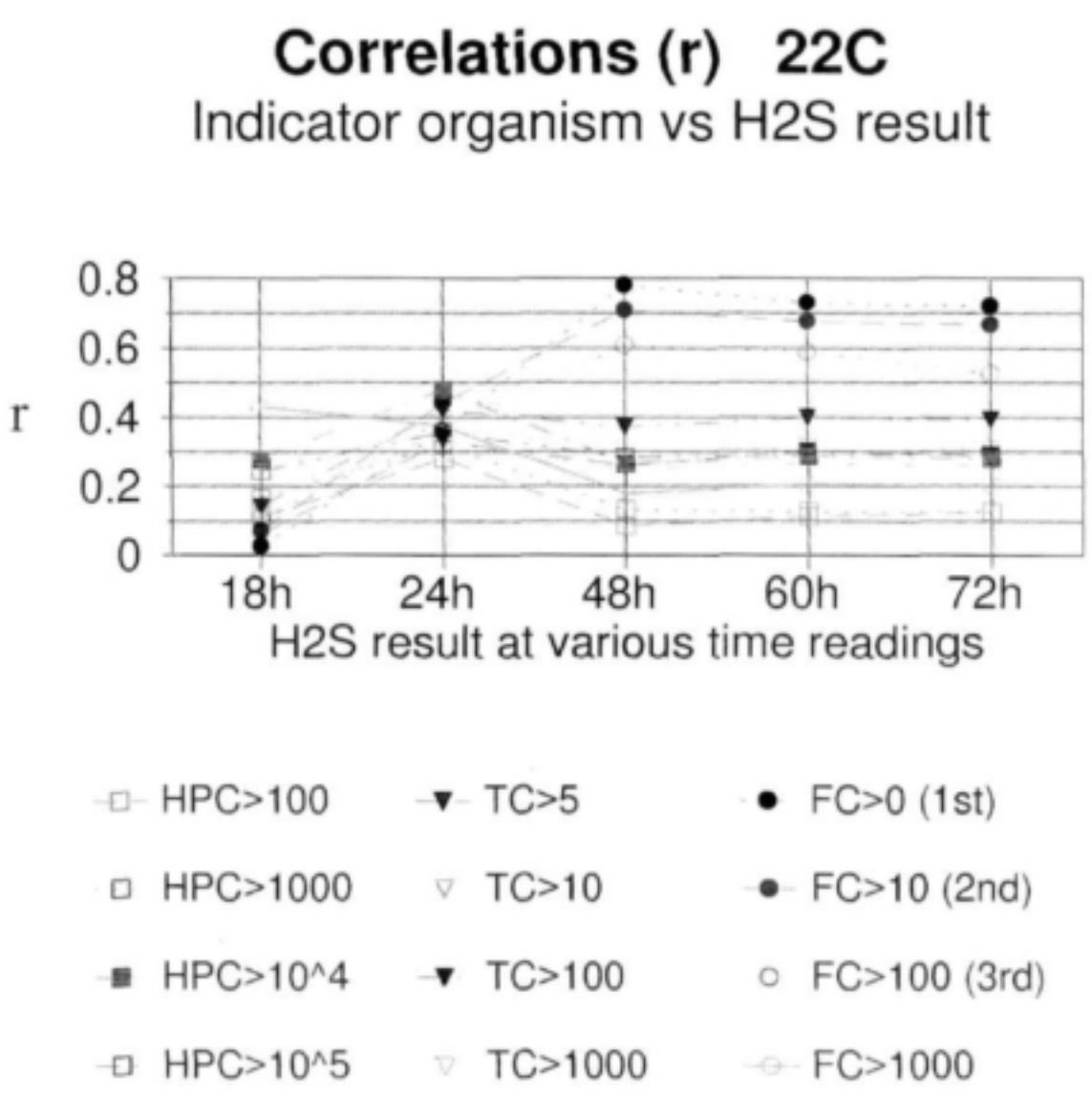


Figure 3.7 Correlations of indicator organisms at different levels vs H_2S test at 22°C (HPC = heterotrophic bacteria; TC = total coliforms; FC = faecal coliforms)

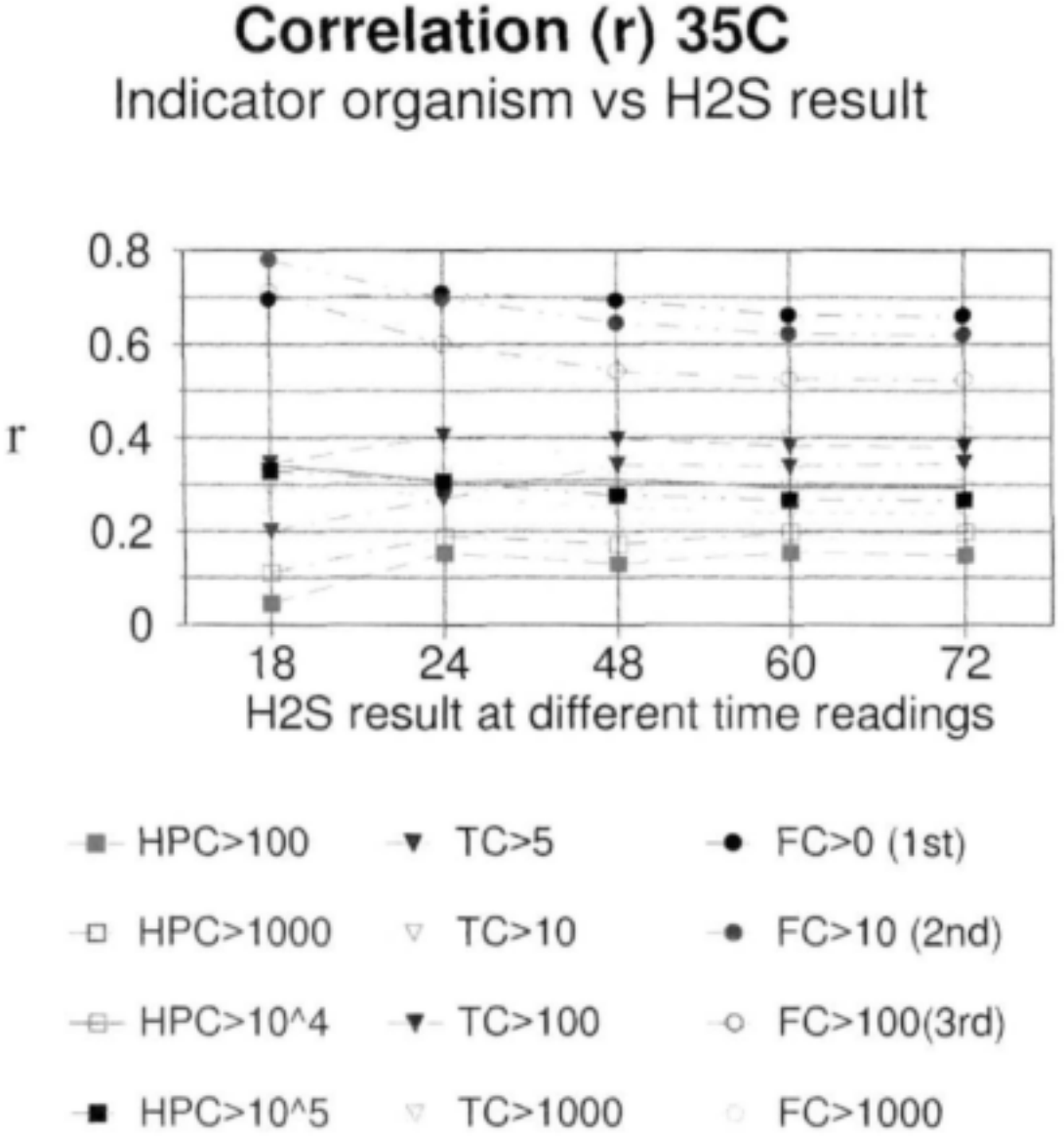


Figure 3.8 Correlation of indicator organisms at different levels vs H_2S test at 35^0C (HPC = heterotrophic bacteria; TC = total coliforms; FC = faecal coliforms)

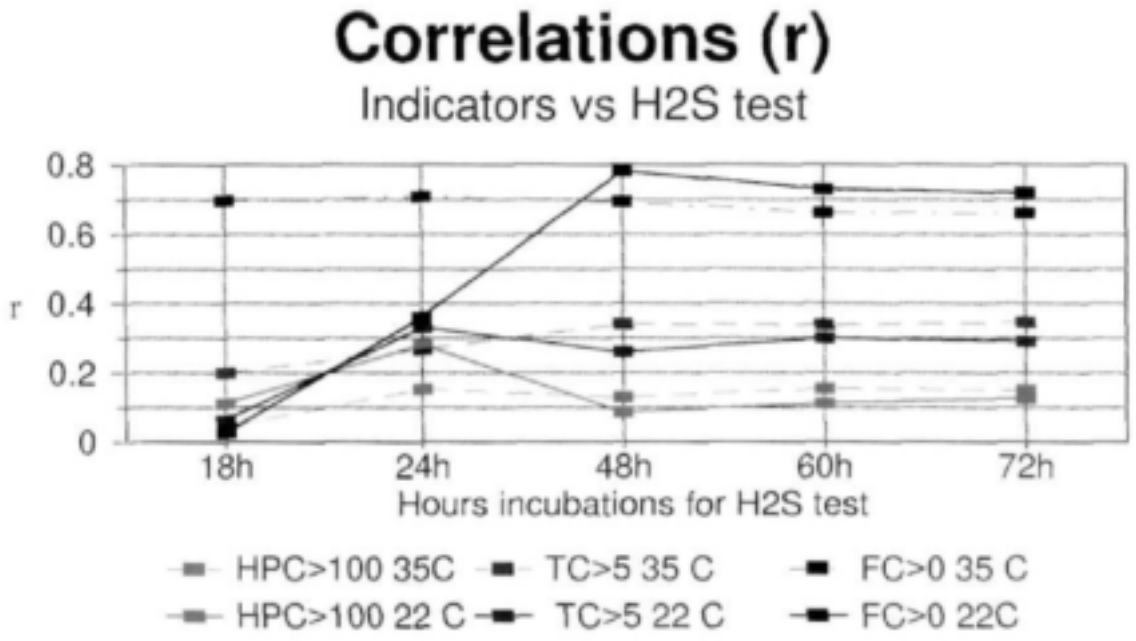


Figure 3.9 Correlations of indicator organisms vs H_2S test using indicator organism data classified as level 1

(HPC = heterotrophic bacteria; TC = total coliforms; FC = faecal coliforms)

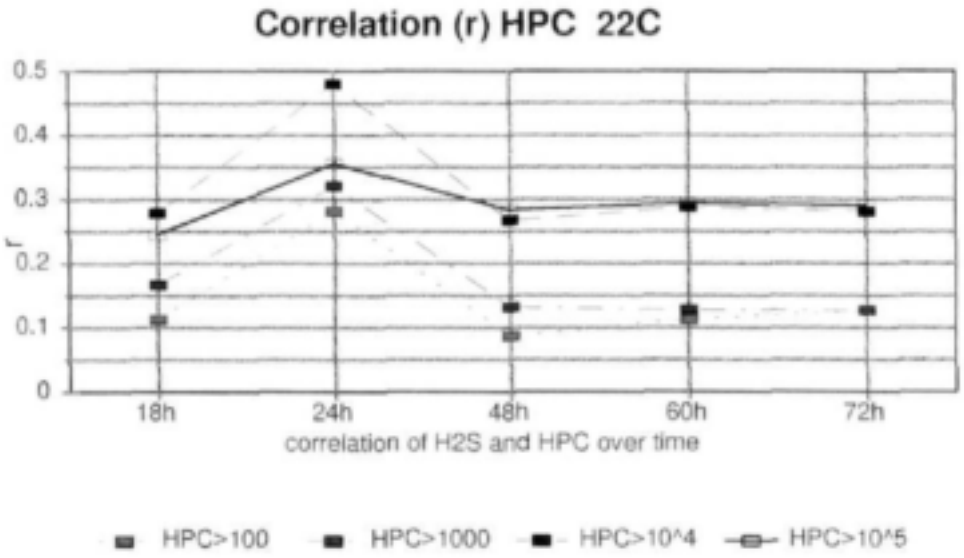


Figure 3.10 Correlation of HPC (heterotrophic bacteria) at different levels vs H_2S test at 22°C

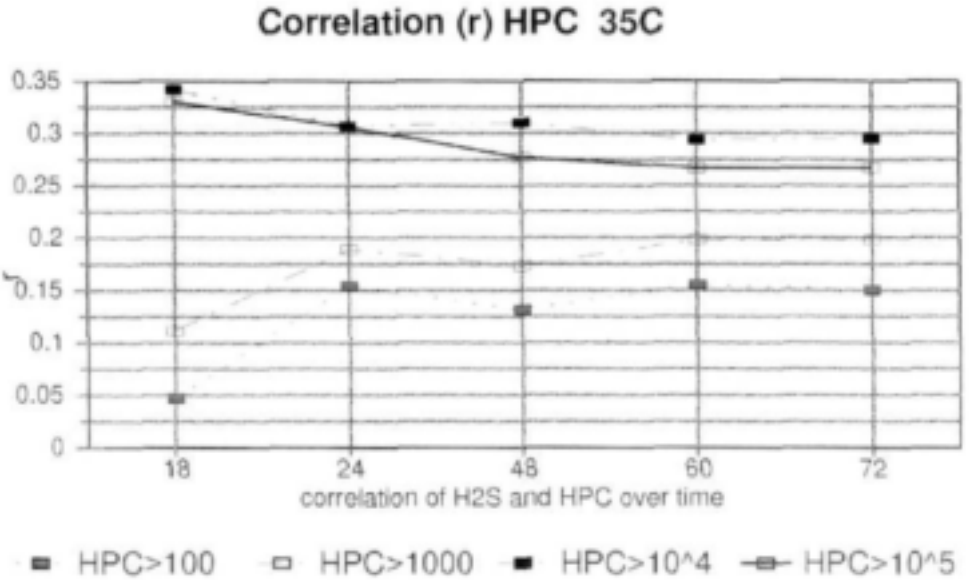


Figure 3.11 Correlation of HPC (heterotrophic bacteria) at different levels vs H_2S test at 35°C

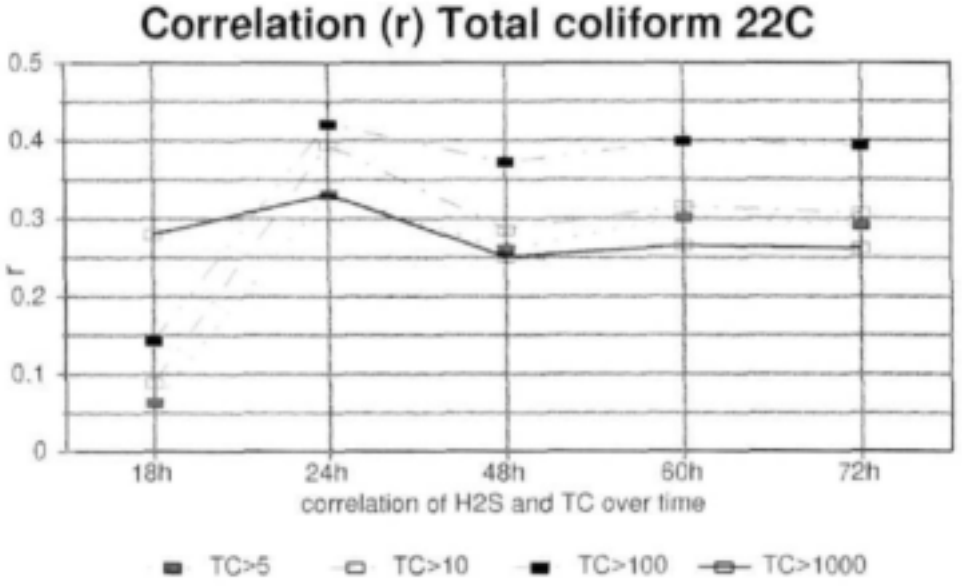


Figure 3.12 Correlations of total coliforms at different levels vs H_2S test at 22°C

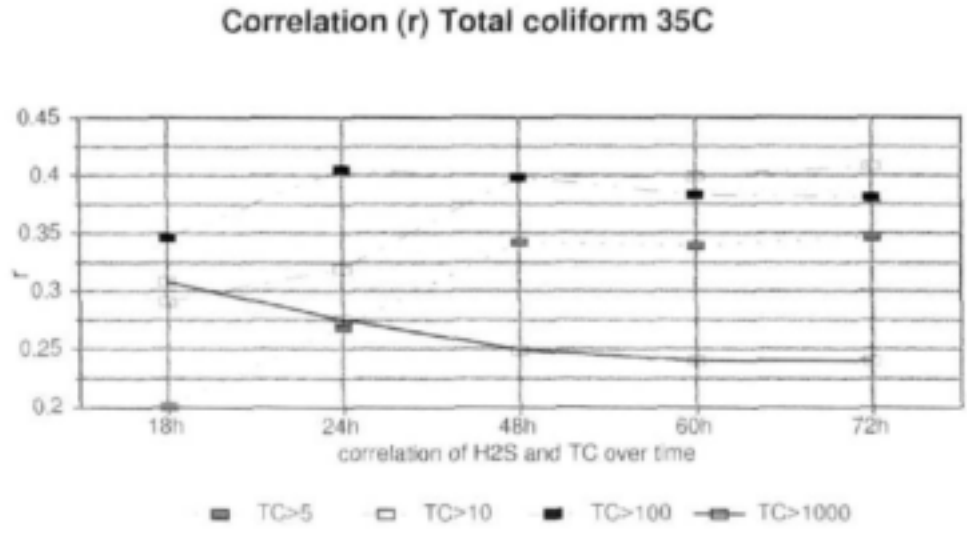


Figure 3.13 Correlation of total coliforms at different levels vs H_2S test at 35°C

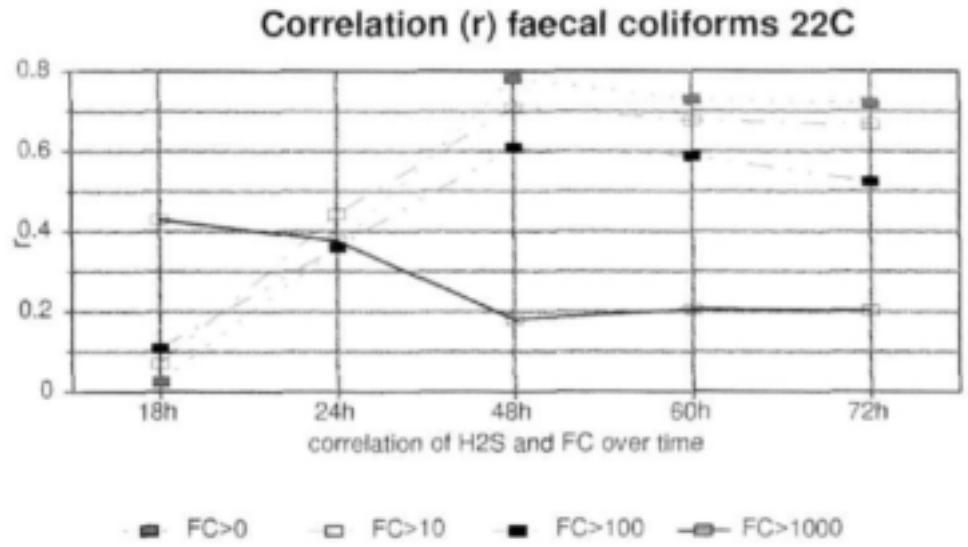


Figure 3.14 Correlation of faecal coliforms at different levels vs H_2S test at 22^0C

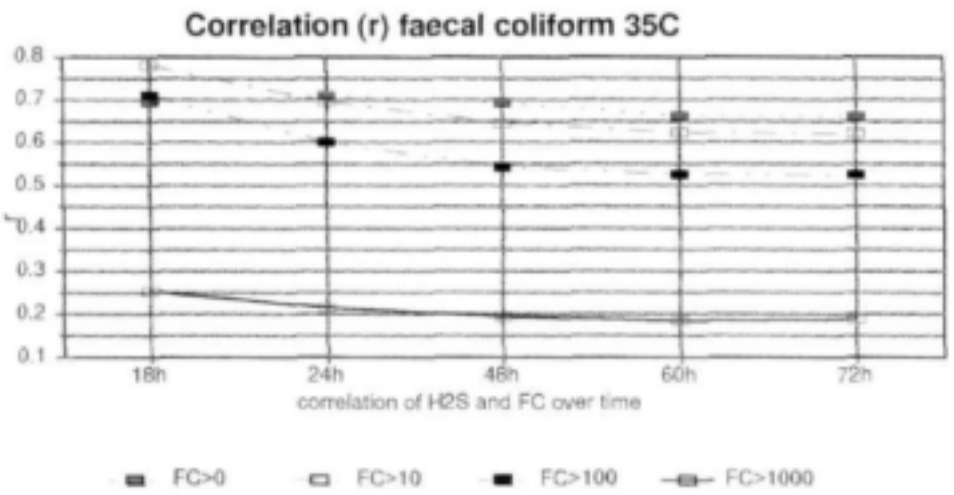


Figure 3.15 Correlation of faecal coliforms at different levels vs H_2S test at 35^0C

4. DISCUSSION

The H_2S strip test was found to be very sensitive in assessing drinking water quality. The strip test was able to detect an average of 2 organisms in a 20 ml sample of water. Sensitivity of the test was not impaired by the presence of high concentrations of non-H_2S producers (10^6 organisms /20ml) (Tables 3.2 & 3.3). A large variety of environmental water samples were included in this investigation, ranging from samples with very low levels of indicator organisms to samples with more than 10^5 heterotrophic bacteria per ml or more than 1000 total and faecal coliforms /100ml.

The correlations between the H_2S strip test and heterotrophic bacteria ranged from r = 0.15 - 0.34 [ignoring results after only 18 hours incubation] although these were all considered to be statistically significant at the 95% level (Appendix 1 Tables A2-A5; where p values were all less than 0.05 with one exception). Similar results were obtained when comparing the H_2S strip test and total coliforms. R values ranged from 0.14 - 0.42 and all were considered to be statistically significant at the 95% level. The faecal coliforms had the best correlation with the H_2S strip test, with r values between 0.19 and 0.79, and all values being statistically significant at the 99% level. Venkobachar *et al, (*1994) also found better agreement between faecal coliforms and the H_2S strip test than between total coliforms and the H_2S strip test. Gawthorne *et al* (1996) found good correlation between the H_2S strip test and the presence of *Salmonella species*.

The highest correlations were found between the H_2S strip test and faecal coliforms at 22°C. This is the most significant aspect of this investigation as it has numerous implications. Firstly, this implies that the test can be carried out in the field. Secondly that the costs to carry out the test will be low in comparison to the traditional indicator organisms as no expensive laboratory equipment is required, and trained but unqualified personnel will be able to conduct the test. The method is a portable one consisting of a small sample bottle (of approximately 40ml volume) containing a sterile paper strip impregnated with the test reagents. The test bottles can be transported easily. An additional benefit of the H_2S strip test is that the reagents and sample bottles can be stored at room temperature without deteriorating, thereby increasing the portability of the test. The reagents have a reported unlimited shelf life (Venkobachar *et al*, 1994) although this has not been tested in this investigation.

The aims of the investigation, as specified in the original proposal, were threefold, namely, to test a novel H_2S strip method for its suitability as a water quality indicator; to test the method for its suitability for detecting contamination of water supplies without requiring incubation at 37°C; and to test the H_2S strip method as a field kit for assessing the water quality of small community water supplies.

The aims have been addressed with the H_2S strip test being found to be

- suitable as a water quality indicator;

- able to detect contamination without incubation at 37°C;
- light, convenient, portable and not requiring storage in refrigerated conditions, making it suitable as a method for assessing water quality of small community water supplies.

5. CONCLUSIONS

The H_2S strip test is a sensitive test capable of detecting low numbers of H_2S producing organisms in a 20ml sample. Non- H_2S producing organisms do not appear to interfere or inhibit the sensitivity of the test. The H_2S strip test was found to have good correlation and 'agreement' with faecal coliform counts.

Results of the H_2S strip test best correlated with indicator organism levels when left to incubate for 48 hours before reading the result, when 22°C was used as an incubation temperature. When incubation was carried out at 35°C the results could be reliably read after 24 hours incubation.

This indicates that the test can be used as a field test or 'on-site' test with many associated benefits. Water quality in small communities and rural environments could be screened on a regular basis, allowing for an improved drinking water quality management programme in general. If water is found to fail the initial test, remedial action could immediately be taken and further analyses could be arranged where the traditional indicator organisms are analysed in an appropriate laboratory. The costs of analysis is relatively inexpensive which would allow more samples to be tested in the monitoring process.

It is not suggested that the H_2S strip test be used as a replacement to the current water quality indicator organisms, but rather that it can be used in addition to those tests, particularly in areas where water quality testing would not have normally been carried out.

6. FURTHER RESEARCH AND RECOMMENDATIONS

Further research is required testing the method under field conditions to determine the effect of factors such as varying and extreme temperatures; sunlight and incubation times. To be able to use the H_2S strip test in the proposed remote areas further research is required. Research will need to confirm that the test is suitable for these remote areas and that it has been adequately tested in the field to allow an endorsement for the use of this test to be made. The method also needs to be tested with field workers and environmental health officers for its use in managing small water supply systems, and testing the attitudes of communities regarding the applicability of the test.

7. TECHNOLOGY TRANSFER

Preliminary transfer of technology of this study is already taking place through interactions with organisations involved in water quality management and analysis in rural communities. For instance, the test is currently being evaluated in the field in the Northern Cape with PD Toens, in the Eastern Cape with Rural Support Services and in Gauteng/ Northern Province with Sego-Dolo Development.

Each participant has agreed to provide feedback on the usefulness of the test and their experiences with it. The instructions describing the methodology sent to the various groups participating in the field testing is provided in Section 9.

8. COST ANALYSIS

A consideration for recommending this H_2S strip test as a method to be used in rural communities and remote areas is the relative affordability of the test. In addition to not requiring laboratory equipment such as incubators and autoclaves, the cost of the individual tests is also relatively low.

In comparison to other water quality indicator tests the H_2S strip test is inexpensive. For example

- the "Defined Substrate Technology" tests such as Colisure (Millipore) or Colilert (Environetics) costs in the order of R30 - R50 per test, or
- the membrane filtration method for faecal coliforms costs ~ R7-00 per test for materials only. The time involved in preparing the media, and carrying out the filtration of the water sample under sterile conditions is not included in this calculation
- the **H_2S strip test costs <R5-00 per test**. This cost also does not take into account the time needed to prepare the sample reagents.

9. INSTRUCTIONS PROVIDED FOR FIELD TESTS

H_2S Strip Test methodology

Introduction:

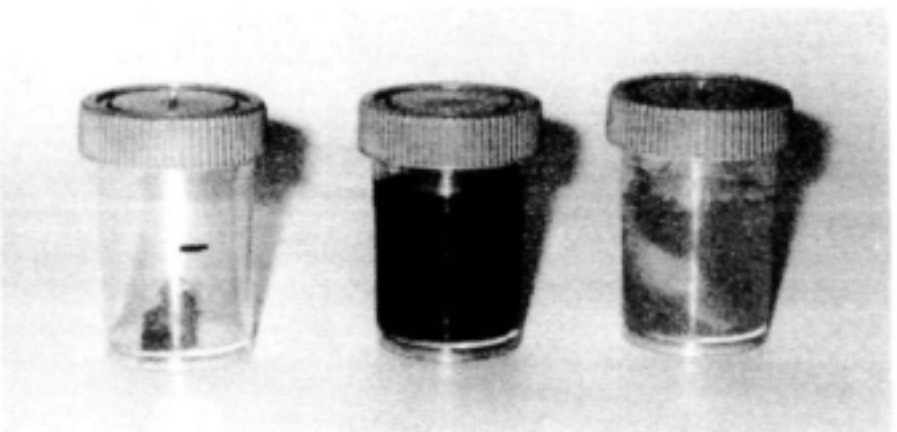
The H_2S strip method is used as a field kit to assess water quality. The H_2S strip method detects hydrogen sulphide producing bacteria.

The H_2S strip method was developed for testing water. It is a simple, affordable, on-site method, which has >80 % correlation when compared to faecal coliforms normally used to assess water quality. The method does not require expensive laboratory equipment such as filtration apparatus and incubators.

A positive result using the H_2S strip method provides an indication that the water quality is not suitable to be used as drinking water, and further investigations are recommended.

Methodology:

- Fill sterile sample bottles provided (containing the paper strips) up to the black line (20ml sample volume). The colour of the water will change to a clear brownish solution.
- Keep sample bottles at room temperature.
- Examine daily, up to 2-3 days, for **black colouration**. This is considered to be a positive result (See photo below- centre bottle)
- A negative result is considered to be a lack of black colour development. The sample may appear murky, but if no black colouration occurs, the sample is still considered to be negative. (See photo below - right hand bottle)



Empty sample bottle positive result negative result

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Appendix 1

- Types of water samples included in analyses
- Correlations between H_2S test and heterotrophic bacteria, total and faecal coliforms at various concentration levels at 22 and 35^{0}C

Table A1 Types of Water samples included in study

- chlorinated Drinking water
- fruit farm dam water
- dam effluent
- river water
- farm effluent
- natural spring water
- dairy laboratory water
- fire hydrant water
- municipal drinking water
- borehole - treated water
- borehole- untreated water
- Theewaterskloof dam water
- winelands district water
- guesthouse drinking water
- mineral water
- sea-farms water
- groundwater from cemeteries

Table A2 Correlations (r) (HPC > 100/ml; TC > 5/100ml; FC > 0/100ml *)

0 C	h	Microbial Parameter	r	p
22*0*C	18h	HPC TC FC	0,113 0,063 0,026	0,021 0,19 0,591
	24h	HPC TC FC	0,282 0,331 0,36	0,000 0,000 0,000
	48h	HPC TC FC	0,084 0,260 0,718	0,076 0,000 0,000
	60h	HPC TC FC	0,113 0,301 0,729	0,021 0,000 0,000
	72h	HPC TC FC	0,127 0,291 0,715	0,009 0,000 0,000
35*0*C	18h	HPC TC FC	0,0473 0,201 0,696	0,336 0,000 0,000
	24h	HPC TC FC	0,154 0,269 0,709	0,002 0,000 0,000
	48h	HPC TC FC	0,131 0,342 0,694	0,008 0,000 0,000
	60h	HPC TC FC	0,155 0,339 0,662	0,002 0,000 0,000
	72h	HPC TC FC	0,150 0,347 0,661	0,002 0,000 0,000

* HPC = heterotrophic bacteria; TC= total coliforms; FC = faecal coliforms

Table A3 Correlations (r) (HPC >1000/ml; TC > 10/100ml; FC >10/100ml*)

0C	h	Microbial Parameter	r	p
22◦	18h	HPC TC FC	0,167 0,088 0,072	0,001 0,072 0,146
	24h	HPC TC FC	0,321 0,395 0,443	0,000 0,000 0,000
	48h	HPC TC FC	0,132 0,285 0,709	0,007 0,000 0,000
	60h	HPC TC FC	0,128 0,316 0,678	0,009 0,000 0,000
	72h	HPC TC FC	0,127 0,307 0,667	0,010 0,000 0,000
35◦C	18h	HPC TC FC	0,111 0,291 0,780	0,024 0,000 0,000
	24h	HPC TC FC	0,189 0,319 0,697	0,000 0,000 0,000
	48h	HPC TC FC	0,173 0,398 0,646	0,000 0,000 0,000
	60h	HPC TC FC	0,198 0,399 0,622	0,000 0,000 0,000
	72h	HPC TC FC	0,198 0,408 0,620	0,000 0,000 0,000

* HPC = heterotrophic bacteria; TC= total coliforms; FC = faecal coliforms

Table A4 Correlations (r) (HPC > 10,000/ml; TC > 100/100ml; FC > 100/100ml*)

0C	h	Microbial Parameter	r	p
22°c	18h	HPC TC FC	0,279 0,142 0,108	0,000 0,004 0,028
	24h	HPC TC FC	0,4802 0,421 0,361	0,000 0,000 0,000
	48h	HPC TC FC	0,297 0,372 0,609	0,000 0,000 0,000
	60h	HPC TC FC	0,288 0,399 0,588	0,000 0,000 0,000
	72h	HPC TC FC	0,281 0,393 0,579	0,000 0,000 0,000
35°C	18h	HPC TC FC	0,342 0,346 0,711	0,0000 0,000 0,000
	24h	HPC TC FC	0,307 0,405 0,601	0,000 0,000 0,000
	48h	HPC TC FC	0,310 0,398 0,543	0,000 0,000 0,000
	60h	HPC TC FC	0,294 0,383 0,525	0,000 0,000 0,000
	72h	HPC TC FC	0,295 0,381 0,524	0,000 0,000 0,000

* HPC = heterotrophic bacteria; TC= total coliforms; FC = faecal coliforms

Table A5 Correlations (r) (HPC > 100,000/ml; TC > 1000/100ml; FC > 1000/100ml*)

0C	h	Microbial Parameter	r	p
22◦c	18h	HPC TC FC	0,245 0,279 0,430	0,000 0,000 0,000
	24h	HPC TC FC	0,356 0,331 0,376	0,000 0,000 0,000
	48h	HPC TC FC	0,284 0,250 0,180	0,000 0,000 0,000
	60h	HPC TC FC	0,294 0,265 0,206	0,000 0,000 0,000
	72h	HPC TC FC	0,290 0,261 0,203	0,000 0,000 0,000
35◦C	18h	HPC TC FC	0,330 0,308 0,253	0,000 0,000 0,000
	24h	HPC TC FC	0,306 0,275 0,215	0,000 0,000 0,000
	48h	HPC TC FC	0,277 0,249 0,194	0,000 0,000 0,000
	60h	HPC TC FC	0,267 0,240 0,187	0,000 0,000 0,000
	72h	HPC TC FC	0,267 0,240 0,188	0,000 0,000 0,000

* HPC= heterotrophic bacteria; TC = total coliforms; FC =faecal coliforms

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