THE HYDROGEN SULPHIDE (H_2S) PAPER-STRIP TEST

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February 2005 *SOPAC Technical Report 373*

THE HYDROGEN SULPHIDE (H_2S) PAPER-STRIP TEST

A SIMPLE TEST FOR MONITORING DRINKING WATER QUALITY IN THE PACIFIC ISLANDS

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February 2005 *SOPAC Technical Report 373*

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Cataloguing in Publication Data:

Mosley, Luke M.
The hydrogen sulphide (H_2S) paper-strip test: a simple test for monitoring drinking water quality in the Pacific Islands/Luke M. Mosley and Donald S. Sharp. – Suva: SOPAC, 2005.

21 p.: ill.; 30 cm
ISSN: 1605-4377

1. Water quality – testing
2. Water quality – Pacific Islands
I. Sharp, Donald S.
II. SOPAC Technical Report 373
III. Title

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SUMMARY

Many people living in the Pacific Island region are reliant on drinking water from shallow groundwater lenses and streams, or from roof catchment systems. These water resources are often scarce and vulnerable to contamination from poorly-installed sanitation facilities (Falkland 1999). The majority of the Pacific's population therefore is at risk to water-borne diseases (Prüss et al., 2002). Many islands are too small in size and under resourced to support conventional water treatment facilities and distribution networks. In urban areas, distribution and treatment systems are reasonably common although maintaining the systems in good working order is sometimes difficult. People living in rural areas and outer island groups are most at risk of contracting water-borne diseases, as they do not typically have access to treated water.

Micro-biological and chemical testing of drinking water quality should be performed to indicate whether water is safe to drink. Unfortunately, in many Pacific Islands the infrastructure needed to adequately monitor water quality is either non-existent or inadequate. A lack of monitoring is particularly apparent for outer islands and rural areas.

Typically, the key water quality parameter that indicates safety is the absence of faecal coliform bacteria in the sample. These bacteria indicate the probability of pathogens (e.g. typhoid and cholera) being present. Sophisticated and costly equipment is required to test for these organisms; i.e. an incubator, filtration apparatus, and chemical reagents, which must be stored under refrigeration. The cost of one test can be more than NZ$30, depending upon the type of test and method used. In addition, samples for coliform analyses must be kept chilled and delivered to the laboratory within 6 h. in order to adequately preserve the sample. In all cases, the time elapsed between collection and examination should not exceed 24 h (APHA, 1995). Although there are several commercially available portable kits that make it possible to carry out on-site water quality testing, these are usually costly and require technical expertise to operate (Bartram & Balance 1996).

An alternative low-cost test for faecal contamination in drinking water which is simple to use and easy to interpret is the *hydrogen sulphide (H_2S) paper-strip test* (Manja et al. 1982).

AIM OF THIS REPORT

The aim of this report is to provide information on the scientific basis, manufacture and use of the H_2S paper strip test in the Pacific Islands. Suggestions are given on how the test could be utilised for rural, outer island and community-based water quality monitoring.

INDICATORS OF FAECAL POLLUTION

Untreated or improperly-treated drinking water may contain micro-organisms of faecal origin that are pathogenic (disease-causing) such as those that cause cholera and typhoid fever. The presence of pathogens in drinking water is usually due to human and animal waste entering the water source. The sanitation facilities that are used predominantly in rural/outer islands of the Pacific are septic tanks and pit latrines, which do not provide sufficient treatment to remove pathogens. The waste outflow from these types of facilities, in certain soil conditions can travel several hundred metres underground (Dillion 1997). Animals (e.g. pigs and cows) in the area of unprotected water supplies can also cause serious contamination and pose risks to public health.

It is difficult and expensive to test for the pathogenic organisms that may be present in contaminated drinking water. Therefore indicator organisms are used to determine the *risk* that these organisms might be present in drinking water. Indicator organisms are always present in high numbers in faecal material, whether or not pathogenic organisms may be present. A high level of indicator organisms in a water sample *indicates* a high risk that pathogenic organisms might also be present. The most common indicator organisms used to determine bacteriological water quality are total and faecal coliforms.

The coliform group of bacteria, along with many other naturally-occurring bacteria, inhabit the intestinal tract of warm-blooded animals, including humans, and are discharged in their faeces. Faecal coliform presence indicates that water is contaminated with faecal matter and is not safe for drinking purposes. In the tropics, coliforms are not an ideal indicator as they can occur naturally and reproduce in soil and water at the ambient temperatures (WHO 1996). Other indicator organisms are sometimes used which are in the *Enterococcus* bacteria group, such as faecal streptococci (WHO, 1996), and *Clostridium perfringens*. The problems noted above with the sophisticated testing procedures and equipment required for the analysis of the above indicator organisms make their use difficult in rural areas and on outer islands.

Another less commonly used indicator is sulphide-reducing bacteria and the following sections outline the use of a low-cost test for these bacteria in drinking water called the *hydrogen sulphide (H_2S) paper-strip test*. There are many advantages of this test for use in rural and remote Pacific Island communities particularly where conventional monitoring is not possible or too expensive.

BACKGROUND AND SCIENTIFIC BASIS OF THE H_2S TEST

In 1975, Allen and Geldreich showed that the presence of coliforms in water was also associated with hydrogen sulphide (H_2S) producing organisms. In 1982, Manja *et al*. developed a simple paper-strip method to screen for bacteriological contamination of potable waters. This study, and several subsequent studies, have found that the H_2S test gave generally good agreement with the standard Most Probable Number (MPN) and membrane filtration methods commonly used for determining the presence and number of coliform and faecal coliform organisms (Hazbun & Parker 1983; Dutka 1990; Castillo et al. 1994; Martins et al. 1997; WHO 2002). As noted in a multi-country intercomparison study summarised by Dutka (1990), this test is "an ideal tool for testing rural and isolated drinking water supplies".

Bacteria can produce hydrogen sulphide through the anaerobic catabolism of cysteine, an amino acid containing the sulfahydryl group, or by the use of elemental sulphur or some oxidised sulphur compounds as the terminal electron acceptor in their metabolic processes. All members of the *Enterobacteriacae* group are capable of the former while the latter occurs in dissimilatory sulphate-reducing bacteria. The H_2S test uses a medium with thiosulphate as a sulphur source and ferric ammonium citrate as an "indicator," only certain enteric bacteria will produce hydrogen sulphide resulting in the development of a black precipitate. Hydrogen sulphide is produced by the reduction of thiosulphate and then reacts with the ferric salt to form an insoluble black ferrous sulphide precipitate. Members of the Enterobacteriacae group such as *Salmonella, Citrobacter, Clostridia, Klebsiella* and *Proteus* are all able to produce hydrogen sulphide in such a medium. Some other non-gut bacteria can reduce thiosulphate into hydrogen sulphide in anaerobic conditions. These bacteria are not typically present in drinking water. The presence of the iron as an indicator in the H_2S medium, would inhibit some naturally-occurring bacteria from producing hydrogen sulphide.

The Codex Alimentarious Commission recommends the use of sulphite reducing anerobes as an indicator for testing bottled natural mineral water (CAC/RCP 48-2001). A report by WHO (2002) did not recommend the use of H_2S bacteria for routine monitoring of water supplies due to the possibility of false positives from naturally-occurring sulphite reducing bacteria. Nevertheless, these bacteria can be a valuable tool in that their presence show a lack of sanitary protection somewhere within the system and indicate a need for further investigation and/or treatment.

Basics of the H_2S paper strip test

The H_2S test is recommended for testing drinking water derived from surface water, boreholes, and rain water sources for faecal contamination.

The reagents used to make the H_2S paper strip test are common laboratory chemicals. By adding a measured amount of "boiled" water and a common liquid detergent to the reagents, a measured amount is impregnated on a piece of absorbent paper and dried in a low-temperature oven. The dried paper strip is placed in a clear small plastic or glass bottle or tube. A water sample is collected in the container containing the reagents and stored in the dark at room temperature for about 3 days. If the sample contains hydrogen sulphide producing organisms, the pad and water turn black. The black colour and the

[1] The test is not currently recommended for use in testing seawater

rotten egg smell of hydrogen sulphide clearly indicate that there is a problem. With such an indicator it is not difficult to convince uneducated villagers that the water may not be safe to drink.

Advantages of H_2S test for use in the Pacific Islands

The advantages of this test over other more sophisticated analyses like the total and faecal coliforms (membrane filtration and Most Probable Number) methods are:

1. Low-in-cost, the cost of reagents for one test is estimated at NZ$0.08 (see Appendix 1). All other materials used can be found locally.
2. When making up and using the test it is not necessary to have access to a laboratory or expensive equipment like an autoclave or incubator. Only a simple balance to weigh the media, pipettes, and a method of sterilizing the kits (hot oven, autoclave, UV light) are needed.
3. Does not require samples to be shipped or stored under refrigeration.
4. Samples are incubated at room temperature.
5. Very easy to use in the field as it consists of only a sample tube.
6. Simple for non-technical people to understand as a clear colour change is observed.

For these reasons the test can be distributed to households/communites so they can test their own water. With sufficient public education on the test, there should be no need to go back and tell them that their water is safe or contaminated as they are conducting the test themselves. If results indicate high risk, households would be instructed to treat their water to make it bacteriologically safe before drinking.

Disadvantages of H_2S test for use in the Pacific Islands

1. Sulphide-reducing bacteria (responsible for production of H_2S) are common in the intestinal tracts of most animals making them good indicators for faecal contamination. However, there are some bacteria within the group that occur naturally around thermal vents, vegetation undergoing bacterial decomposition, etc., which may yield a false positive test result (WHO 2002). For most drinking water supplies and all rainwater cistern systems, such ‘false positives’ would not be expected. As mentioned above there are members of the coliform group that are naturally occurring as well.
2. Some H_2S-producing bacteria such as *C. perfringens* are spore formers and hence they may be present long after a pollution episode has occurred (WHO, 1996). However, pathogens may also survive for long periods of time in the tropics (Dillion 1997).
3. Another criticism of the test has been its use as a presence/absence test. The number of indicator organisms in a water sample can indicate the degree of contamination and therefore relative risk to public health. The H_2S test just indicates whether there is a risk, not the degree of risk. However, the speed of the reaction (color change from clear to black) indicates bacterial density. The faster the reaction, the greater the numbers of organisms present. If necessary, estimates of the concentration of bacteria of faecal origin can be made by controlling the volume of the sample used, or by using a three-tube or five-tube series Most Probable Number method.

MAKING THE H_2S PAPER STRIP TEST

1. Any type of glass bottle or plastic tube with a volume of between 15-200 ml, which has a heat resistant cap/lid, can be used. The bottles or tubes are first cleaned by washing in detergent, rinsing with tap water, soaking in a 5% bleach solution overnight and rinsing with tap water and drying in air or in an oven.

2. If no volume marks are present on the bottles, they can be marked at 10 ml, 20 ml or 100 ml volume, or any volume in-between – depending upon the bottle size. The authors typically use bottles marked at a 10 ml volume. This volume calibration is typically done by measuring the required (i.e. 10, 20, 100 mL) of water into a graduated cylinder or other measuring device, pouring the measured volume into a sample bottle, standing the bottle upright and then making a mark on the bottle where the water level is. By lining up this bottle with another bottle, the other bottles can be marked in approximately the same place. A glass marking pencil, permanent ink pen or tape can be used to mark the desired volume.

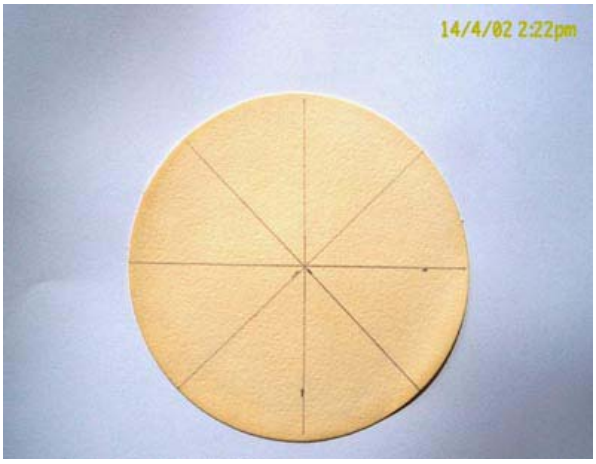
3. The medium used in the test is prepared from the following chemicals, which are dissolved into distilled or boiled tap water. Shake or stir the mixture to dissolve the chemicals.

H_2S Media Formula

Bacteriological peptone	40.0g
Dipotassium hydrogen phosphate	3.0g
Ferric ammonium citrate	1.5g
Sodium thiosulphate	2.0g
Citrate (optional but increases sensitivity	g?
Liquid detergent (e.g. Teepol)	2.0ml
Water (distilled or boiled tap)	100.0ml

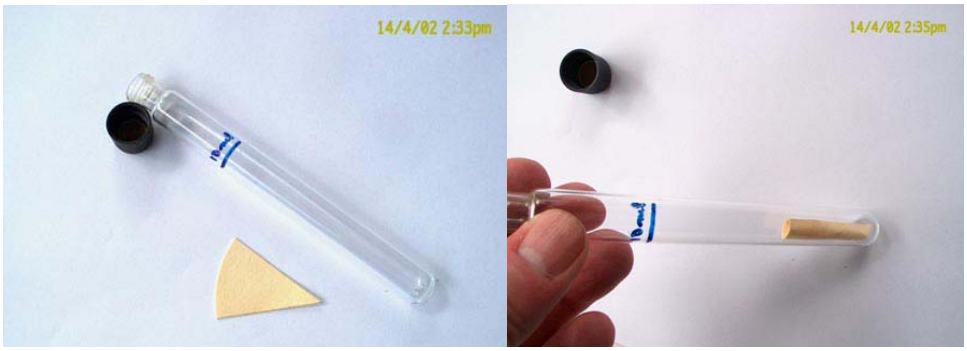
4. Taking absorbent paper, filter paper, non-toxic paper towelling, gauze, absorbent pads used for membrane filtration, or any other type of absorbent material[2], place a measured quantity of media onto the paper. Each paper strip for a 10 ml test sample needs to contain 0.5 ml of media (50 ml sample will use 1 ml of media and a 100 ml sample will require 2.5 ml of media etc[3]). Large adsorbent pads can be cut to a size that has absorbed 0.5 ml of media. For example in the picture below, the pad is ready to be cut into eight paper strips, therefore 4 mL of the media will have been poured onto the pad (0.5 mL per strip).

[2] Coasters used in bars work well (if no black ink is used).
[3] A pipette graduated at 0.1 ml intervals may be necessary for this.



5. The next step is to dry the strips in an oven at about 55°C. A conventional household oven on low temperature can be used. These reagent-impregnated strips can be stored dry (in an envelope or preferably a zip-locked bag) for several months – until ready for use.

6. Before conducting the test, a strip or strips are introduced into the appropriate (clean) sample bottle.



7. Bottles should next be loosely capped and sterilized by various possible means:

- Plastic and/or glass tubes can be placed in a hot air oven at about 120°C for 60 minutes.
- If the tubes are clear plastic they can be sterilized under UV-light for at least 30 minutes.
- If the tubes are autoclavable-glass (pyrex), sterilization can be done with an autoclave for 30 minutes.
- Tubes can be placed in a simple pressure cooker for 15 minutes at 115°C.
- It can also be done by steam (in a rice steamer) for about 30 minutes.

Sterilisation in a hot-air oven

Following any of the heat treatments, the tubes or bottles are then allowed to cool and the caps or lids tightly sealed. The tubes or bottles should be stored in a dark place until ready for use. Experience has shown they can be stored for at least 5 years in this manner.

Alternatively, the media can be prepared in pre-weighed, dry form and stored until ready for use. A central laboratory could make the impregnated pads (filter paper) and provide these in sealed plastic "zip-locked" bags or envelopes, or provide ready-made tubes or sample containers. In dry form, these reagent-impregnated pads can be stored indefinitely. The only step required of the community is to insert the reagent coated paper strip in sample containers 15-200 ml in size, and sterilize.

Sampling Procedure

A basic instruction sheet for distribution to individual households on sampling and interpreting results is shown in Appendix 2. The following procedures are for conducting a survey of water quality using the H_2S test.

1. At the time of sampling, label each container with a sample number.

2. Write the sample number, date and time of collection on the special report form (see Appendix 3). Include on the sheet additional information on the type of water sampled (well, surface, rain water, treated supply, etc.) and exact location of collection, such as "at the tap nearest the borehole." Under remarks note if the water was visibly turbid or has any other characteristic that should be noted.

3. At the bottom of the report form there is space reserved for "Notes." Record any observations that may have influenced the quality of the water sampled. For example, distance to a nearby source of pollution, faulty pump, or state of sanitary protection (if a well or spring).

4. Standard procedures specify that if the sample is from a tap, flame the mouth of the tap to eliminate the chance of accidental contamination (a false positive), then, let the water run freely for about 15-20 seconds. Place the opened H_2S sample

collection bottle under the tap and collect the appropriate amount (e.g. up to 10mL calibration mark) being careful not to contaminate the cap. It should be noted however that samples should not be collected from taps that are leaking and flaming the tap is not necessary if you are testing the quality of the water as it is actually consumed.

5. If the water to be sampled is from a storage container, tank or cistern; a natural flowing water body like a spring or stream; or from a dug well, use the utensil that is normally used by the consumer or water collector to collect the sample, rinsing it several times before collecting the sample.

6. Every day of sampling, a *control* sample should be collected and analysed. This is a sample that is known to be uncontaminated, such as boiled water, commercially bottled water, or water treated with chlorine. The control sample is used as a benchmark to compare color change in the test samples and to ensure that the sample bottles have been properly sterilized prior to use *(Note: There will be slight change in the color of the sample to a pale yellow or light brown due to the color of the reagent, which is normal).*

READING AND INTERPRETING RESULTS

1. After sampling, place all test samples in a dark place and incubate at room temperature for a total of three days. Every 12-18 hours examine the samples for changes in color. The date and time of each observation is recorded on the report form and the observations are recorded as follows: (–) = no change; (+) = slight change, the paper strip or water has turned gray; (++) = the paper strip is partially black; (+++) = the strip and the water sample itself are noticeably black.

2. As noted above, a color change indicates the presence of bacteria of faecal origin. The speed of the reaction will determine the density of organisms present; i.e. the quicker the reaction the higher the number of faecal organisms presence. This can also be interpreted in terms of a risk factor. For example, a slight color change (+) on day three indicates a lesser risk than a strong (+++) change on day 1.

3. To determine actual risks to health, H_2S test results must be considered in parallel with the results of a sanitary survey. An example of a sanitary survey form for rainwater tanks is shown in Appendix 4. For example, if a drinking water well is unprotected and the results of the H_2S test are positive on the first day, the users should be informed that a risk to health is likely, and steps must be taken to disinfect the water. Usually after seeing the results first hand, the user understands that the water supply in question is not suitable for drinking purposes. In such cases the users are generally receptive to taking corrective action; i.e. to protect the well from contamination, or to learning about *disinfection* (treating water to kill bacteria).

   Various household disinfection techniques could be recommended such as boiling, adding a few drops of chlorine bleach (4 drops per litre), and/or putting the water in a clear plastic bottle and exposing it to full sunlight for a minimum of 4 hours (e.g. 10:00am-2:00pm). Two H_2S tubes could be distributed to each household, with instructions to fill one with untreated water and the other with water that has been treated. After the 2-3 days of incubation, no color change should occur in the treated sample, which clearly shows that the organisms that caused the untreated sample to turn black have been deactivated.

HOW THE H_2S TEST CAN BE USED

This low-cost test has several useful functions[4].

This test can be used:

1. For monitoring of rural and outer island water supply systems where it may be difficult to conduct conventional testing due to a lack of appropriate laboratory facilities. This, along with sanitary survey data (information identifying conditions that may lead to the contamination of a water supply source) would make it possible for communities to monitor their own water supplies without having to rely on central laboratory services. If community-based programmes of this nature can be established, outer islands could carry out their own water quality monitoring and surveillance programmes and initiate corrective action when needed. This would help to protect public health in these areas and result in a substantial cost savings to island governments.

2. For routine monitoring of reticulated systems; i.e. water that is distributed through a piped system. If a positive result is observed, another sample can be collected for further analysis by conventional means e.g. for faecal coliform enumeration.

3. To determine the cleanliness of water storage tanks, rainwater cisterns and other household storage containers.

4. To identify sources of contamination or the point in a reticulated system where bacteriological contamination is being introduced.

5. To select which spring is best to develop.

6. To determine effectiveness of disinfecting a water source, or to verify that a well has been properly protected.

7. As a tool in health and hygiene education to show villagers how water becomes contaminated and what they can do about it. Communities would also have evidence to alert the relevant authorities that water, which is supposedly treated, is still contaminated.

8. For monitoring during emergencies and disasters such as cyclones where conventional testing is difficult. For example, following a cyclone, thousands of kits could be locally manufactured and distributed in a short time period (few days) to individual households by community health workers along with printed material on their use. As most water sources on outer islands are localized small community or individual sources, time and resources could be focussed on distribution of the kits to the maximum number of people. This would mean that many more drinking water supplies are tested compared to if conventional methods were used by a water or health agency. This should enable better protection of human health following disasters.

9. To identify sources of pollution entering streams and rivers by comparing differences in incubation times of samples collected at regular intervals along the stream or river.

[4] Keep in mind that this is a tool to illustrate bacteriological contamination and is not a standard method that is admissible in legal proceedings. The major usefulness of this test is it's application as a low-cost educational tool.

10. To demonstrate how easily hands become contaminated and how easily they can contaminate food and water. For example, it can be used to demonstrate the effectiveness of washing hand with soap; i.e. to illustrate the faecal oral route of disease transmission. This is done by pouring clean (boiled and cooled) water over unwashed hands and testing it, and having others wash their hands with soap and repeating the exercise.

11. To determine if a food contact surface is contaminated with an a common food-borne pathogen like *Salmonella* by swabbing the surface with a sterile swab and inserting the swab in an H_2S tube containing sterile water.

Commercial availability of H_2S test

Although the test is very simple to make and this should be possible in most countries, the test may also be purchased when manufacture is not possible.

1. H_2S tests or chemical reagents may also be able to be obtained on a 'cost of manufacture only' basis from the

Institute of Applied Sciences,
University of the South Pacific,
Box 1168,
Suva,
Fiji Islands.

pH: (679) 3212967 or 3212965
Fax: (679) 3300373
See website for email contact details: www.usp.ac.fj/ias

2. HACH chemical company make a H_2S test called the *Pathoscreen* test.

VALIDATION OF H_2S TEST FOR USE IN THE PACIFIC ISLANDS

The H_2S test has been used in several Pacific countries but no detailed comparisons have been published where this test method is compared against other methods of assessing the microbiological quality of water. For this reason we undertook laboratory and field testing and validation procedures to ensure the test was suitable for use in the Pacific Islands.

Laboratory Testing

In order to determine how this test can be developed for use in developing countries, a series of experiments were conducted on three naturally-occurring water types which are commonly used as drinking water sources:

1. Water from a large river (currently used as a water supply) flowing through a rural catchment.
2. Water from a small creek flowing through a semi-urban area.
3. Water from a typical household rainwater cistern system found in Fiji.

The intent of these experiments were to determine how well the test correlates with other traditional water quality tests and whether or not the time it takes for a reaction to occur correlates with bacterial density and relative risk. All analyses were undertaken using validated methods in the microbiological laboratory at the Institute of Applied Sciences, University of the South Pacific, Suva, Fiji.

The table below shows the time taken for H_2S development compared to bacteria counts (Colony Forming Units per 100 mL, CFU/100mL) using conventional methods for total (TC) and faecal (FC) coliforms. The time taken for H_2S development is separated into the time taken for intial (grey colouration, +) and final (dark black colouration, +++) colour development. The creek water was the most contaminated water with very high counts of total and faecal coliforms, and this water also took the shortest time to turn black. The rain water took longer (92 h) to turn fully black and had low levels of faecal contamination. We also examined the use of the H_2S method to determine bacteria counts using a Most Probable Number (MPN) method (serial dilutions made of sample and these dilution placed in separate H_2S tubes). The results agreed well with the trends in the other results indicating that, if necessary, the H_2S test could be used to estimate bacteria numbers.

Site	Time for H_2S development	Time for H_2S development	TC	FC	MPN H_2S
	initial colour	full colour	CFU/100 mL	CFU/100 mL	CFU/100 mL
River water	40 hours	59 hours	480000	62	250
Creek water	23 hours	25 hours	3820000	2700000	16000
Rainwater	42 hours	92 hours	490000	1	5

The same samples were tested for faecal streptococci (FS, *Enteroccocci*), Salmonella (2 different types of test) and *Clostridium perfringens*. The faecal streptococci results were similar to the faecal coliforms which is not unexpected as both these indicator bacteria groups would be expected to be found together in faecally-contaminated water. *C.*

perfringens, a definite indicator of faecal pollution, was also found in the river and creek samples but not the rain water. *Salmonella* results were variable with one test showing positive for creek water and one test showing positive for river water. *Salmonella* would be expected to be at much lower levels than the other types of bacteria so the statistical probability of finding it in one portion of the sample and not another would be high.

Site	FS col/100 mL	C. perfringens P/A	Salmonella (BSA) PEA	Salmonella (HEA) P/A
River water	63	yes	Absence	Presence
Creek Water	710000	yes	Presence	Absence
Rainwater	5	no	Absence	Absence

In summary, the best correlation of H_2S colour development time with other bacteria levels was for faecal coliforms, faecal streptococci and *Clostridium perfringens*. This is similar to other studies (see WHO 2002) and indicates the suitability of the H_2S test for testing drinking water for faecal pollution.

Field testing of the Hydrogen Sulphide (H_2S) test following Cyclone Ami

The difficulties in conducting water quality monitoring on remote islands are increased following natural disasters such as cyclones. An evaluation of the H_2S test against the conventional indicator organisms (total and faecal coliforms) was undertaken following the occurrence of Cyclone Ami in the Fiji Islands (*SOPAC Technical Report 374*). The H_2S test turned positive (black) for most samples that had faecal and total coliform levels above the respective WHO guidelines. Only about 11% and 8% of the samples that showed faecal and total coliform bacteria respectively did not test positive in the H_2S test, yielding 'false-negative' results. Similar disparities have been observed in other studies (see WHO 2002a for a summary) and are not unexpected as the coliform tests measure different bacterial groups than the H_2S test. Also the sample volume used in the H_2S test (10 mL) is less than for the coliform-type indicators (100 mL), so the statistical probabilities of finding bacteria will differ. Lastly, there is an increased risk of introducing bacterial contamination when collecting and examining samples for faecal and total coliforms, due to the increased number of handling and filtration procedures, as compared to the H_2S test. This is particularly relevant when using the membrane filtration method in difficult non-laboratory conditions prevalent on outer islands. Upon closer examination of our results we found many of the 'false negative' samples had quite low levels of faecal and total coliform bacteria (e.g. see Labasa water depot sample results in Table 1 of *SOPAC Technical Report 374*). Therefore we believe that in many cases people drinking water that gave 'false negative' results in the H_2S tests would not necessarily be exposed to an increased risk to water-borne diseases.

About 2% and 6% percent of the samples that tested positive in the H_2S test did not have any total and faecal coliform bacteria respectively present. These are termed possible 'false-positives.' Similar findings have been documented in the literature (see WHO 2002 for a summary). This is likely due to the fact that some H_2S reducing bacteria (e.g. *Clostridium* sp.) persist in the environment longer than coliform bacteria. (WHO 2002). It could possibly be due to naturally-occurring sulphide-reducing bacteria being present, but the conditions needed for these bacteria to thrive are anaerobic waters with high organic

matter and sulphate content. None of the waters we sampled fitted this description so we consider these results are unlikely to be false-positives in the sense of a natural H_2S producer being present. In any case, a false positive result indicates a problem, which when used in conjunction with a sanitary survey can provide information that would result in the suspect water either not being used, justify the system being cleaned and disinfected, the supply being disinfected or would suggest additional testing using conventional means. Nevertheless, following a disaster event, any positive test results should be regarded as unsafe for drinking purposes and disinfected.

We conducted a second visit to Vanua Levu, and several of the positive H_2S tests were re-tested for the presence of the spore-forming anaerobic bacteria, *Clostridium perfringens,* which is a strong H_2S producer and an indicator of faecal pollution. The results showed a relationship with the speed of H_2S development. Nearly 50% of the samples returned positive *C. perfringens* results, which indicated faecal contamination had entered into water supplies and/or the reticulation system. About 25% of samples that tested positive with the H_2S test had undetectable levels of *C. perfringens*. However, these were generally the samples where the H_2S test was slow to turn black, indicating that few H_2S producing micro-organisms were present when the sample was collected. Although it is suggested that further research on the quality of drinking waters in the Pacific should be performed to confirm the link between positive H_2S test results and the presence of faecal contamination and pathogens, the results thus far indicate that this test is valid.

CONCLUSION

The test is well suited for testing drinking water supplies for faecal contamination in the Pacific Islands, particularly in remote rural and outer island areas. The significant advantages of the H_2S test compared to other conventional microbiological (faecal and total coliforms) analyses is that it is very low in cost and does not require sophisticated equipment to manufacture or carry out the analyses. H_2S kits can easily be produced in Pacific Island countries where laboratories are often poorly equipped, or distributed by a regional organization, when needed. The results from H_2S tests are visual and therefore simple for people to understand, as a black colour change occurs when bacteria levels in drinking water are high. This enables communities and community health workers with minimum training to safely test their own water supplies. The time the H_2S test takes to turn black shows a correlation with faecal levels so an indication of the risk that pathogenic organisms are present can be obtained.

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APPENDIX 1: COST OF H_2S MEDIA

Recent prices in New Zealand dollars (quoted by Biolab Scientific) for the H2S media

	Cost (NZD)	Cost per gram (NZD)
Peptone 500g	$174.80	0.35
Di potassium hydrogen orthophosphate 500g	$103.60	0.21
Ferric ammonium citrate 100g	$30.20	0.30
Sodium Thiosulphate	$56.99	

According to the formula;

40g peptone	= $14.00
3g Di potassium hydrogen orthophosphate	= $0.63
1.5g Ferric ammonium citrate	= $0.45
2g Sodium Thiosulphate	= $0.22

The total is: $15.30 for enough chemicals to make 200 tests or roughly NZD $0.08 per test.

This does not count the absorbent paper, sample container or lab time and equipment.

We can assume these could be free of charge. We obtain free pre-form bottles from Coca Cola company.

Compare this to the cost of an ampoule of MF-endo or other media, the H_2S test is much cheaper. Also, with the other tests you have real lab time and equipment use involved.

The H_2S can adequately indicate when a risk to human health occurs there is no excuse for not undertaking water testing because of a lack of financial or material resources.

As noted above: the test or chemicals may also be obtained on a 'cost of manufacture only' basis from the Institute of Applied Sciences, University of the South Pacific.

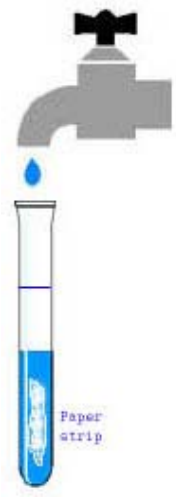
APPENDIX 2

Household Name:.....Phone: .....

Street address/location:.....

Water Quality Testing Using the Hydrogen-Sulphide (paper strip) Test

Good water is essential for health. You can use this simple paper strip test to determine if your water is safe to drink. If the tube turns black within three days of collecting the sample, you must treat your water before drinking it, as harmful bacteria may be present.



When collecting a sample:

1. Allow the tap to run for about 30 seconds.
2. Uncap the lid of the sample tube and fill with water up to the mark.
3. Tightly cap the sample tube and put in a dark place to incubate for 3 days.
4. For comparison purposes, collect a sample of water that you know is safe, like boiled water or commercially-bottled water.
5. Observe the samples every 12 hours (morning and evening) for 3 days and note below if water and/or the paper strip turns black. The water in the tube will have a slight brownish colour immediately after collecting the sample. This is due to the chemicals used and is considered normal.

DAY – 1		DAY – 2		DAY – 3	
TIME IN TUBE	12 hours	24 hours	36 hours	48 hours	60 hours
COLOUR CHANGE NOTED (Y/N)	**Yes/ No**	**Yes/ No**	**Yes/ No**	**Yes/ No**	**Yes/ No**
High Risk		**Moderate Risk**		**Low Risk**	

Note: If the sample tube turns black within 3 days of collection this indicated the presence of bacteria that could cause disease. In such cases the water should be treated before drinking. Treatment consists of boiling, filtering[5], adding chlorine (household bleach), or exposing a 600ml clear plastic bottle of water to direct sunlight for a period of 4-6 hours.

Boiling means bringing the water to a rolling boil for a minimum period of 2 minutes.

Chlorine can be administered by adding 4-5 drops of household bleach to each litre of water treated or by adding chlorine tablets (meant for purifying drinking water) according to the instructions on the box, usually one tablet per litre.

[5] Filtering does not kill bacteria, it only reduces the number and lowers the risk to health.

For further information contact: SOPAC, Ph (679) 338 1377 or watersector@sopac.org

APPENDIX 3

WATER QUALITY TESTING - DATA SHEET

Address: _____

_____

Location of source (describe): _____

RESULTS[2]:

SAMPLE NUMBER	TYPE WATER SOURCE: (Deep well - borehole; shallow well; surface water; spring; etc.)	DATE: of sample collection	TIME: of sample collection	LOCATION: (place where sample is collected)	REMARKS[1]	DAY 1 Date: TIME	DAY 1 Date: TIME	DAY 2 Date: TIME	DAY 2 Date: TIME	DAY 3 Date: TIME	DAY 3 Date: TIME
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											

Notes: _____

_____

_____

1. Indicate under "remarks" if the water is visible turbid, colored, or contains settable solids or material in suspension.
   Also, note any problem at the sampling site like a leaking tap, area unclean or littered, drainage problems, etc.
2. Results: a (-) indicates a negative; a (+), grey color, the reaction has started; (++) the reagent pad is now partially black; (+++) the reagent pad and the water is noticably black.
3. Notes: Indicate the distance between the water source and any sources of pollution, like a compost pit, septic tank, leach field, etc.

APPENDIX 4: RAINWATER CISTERN SYSTEMS – SANITARY SURVEY

Country: _____ **Date of visit:** _____

Atoll: _____ **Island:** _____

Name of community/ village: _____ Est. pop. of village: _____ Est. # (households) in village: _____

Name of family (it is suggested that the tank be numbered for easy identification): _____

Number of people in family that drink water from this tank: _____ *[Note location of tank on map – use same number as sample]*

Sample No.: _____ *[Number that corresponds to number on H_2S sample tube]*

	SCORE
1. Condition of ROOF: good (0)____; fair (1)____; poor (2)____	
2. Condition of guttering: good (0)____; fair (1)____; poor (2)____	
3. Guttering sloped to drain: yes (0)____; no (2)____	
4. Inlet screened or protected: yes (0)____; no (2)____	
5. Interior tank clean: good (0)____; fair (1)____; poor (2)____	
6. Condition of tank: good (0)____; fair (1)____; poor (2)____	
7. Method of withdrawal by tap: yes (0)____; no (2)____	
8. Tap and other plumbing in good repair: yes (0)____; no (2)____	
9. Method for diverting first flush available: yes (0)____; no (2)____	
10. No vegetation overhanging roof catchment area: yes (0)____; no (2)____	
TOTAL:	

OTHER INFORMATION:

1. Type GUTTERING: vinyl ____; PVC ____; metal ____; other [describe]:
   _____
2. Type TANK: fibreglass ____; poured concrete ____; ferrocement ____; PVC ____; galvanized iron ____; other: [describe]_____
3. Location of tank: On raised platform ____; at ground level ____; partially below ground ____; majority of tank below ground ____
4. Estimated capacity of tank, in m^3: _____ ($V=\pi r^2 h$)
5. Date constructed: _____ Date when last cleaned: _____
6. Other method of withdrawal [example, a bucket]: _____
7. Describe method for diverting first flush, if available (sketch on reverse):

RECOMMENDATIONS:

_____

_____

_____

1. H2S test (score): negative (0)____; positive, day 1(11)____; day 2(9)____; day 3 (3)____
2. Sanitary Survey- Risk Score: _____

Relative risk = 1+2: **LOW** (<5)_____; **MODERATE** (5-10)_____; **HIGH** (>10)_____