Hydrogen Sulphide Test Strips and the Detection of Groundwater Contamination by Septic Seepage

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ABSTRACT

The H_2S test strip method is being advanced for microbiological water quality testing in situations where conventional analyses are impractical or too expensive. It involves ambient temperature incubation of water samples with nutrient test strips formulated to generate hydrogen sulphide when 'faecal' bacteria are present. Recently a WHO review (Sobesey & Pfaender 2002) identified several concerns including the limited number of comparative studies, formulation variability, and false positives and negatives. In response to the WHO review we compared the H_2S test's ability to detect and quantify faecal contamination in an aquifer impacted by septic leachfields with data obtained concurrently using conventional analytes - *Escherichia coli*, Enterococci, *Clostridium perfringens*, somatic and F-specific coliphages, faecal sterol biomarkers, *Cryptosporidium* and *Giardia*. Like other analytes, H_2S testing detected the steep contamination gradient ranging from high (septic liquid) to moderate (exfiltration zones), to background (domestic bores), corresponding to indicator reductions/dilution >99.9999%. Single plus/minus tests were unable to distinguish between heavily and slightly contaminated waters. Multi-tube testing, especially using 10 X 10 mL arrays, however, allowed the pollutant gradient to be detected. It was concluded that while the WHO review concerns are justified, the H_2S test performance shows promise in sanitary survey work and can be improved by employing a multiple tube/ mpn approach and has potential for the protection of source water and identifying contamination.

INTRODUCTION

The H_2S test strip method is being advanced as a technique for acquiring microbiological water quality data comparable to coliform testing for users and managers where conventional tests are either impractical or too expensive for the community serviced. Typically it involves room temperature incubation of 10-100 mL water samples containing a nutrient test strip formulated to detect generated hydrogen sulphide where 'faecal' bacterial are present. Given perceived cost and infrastructure savings the World Health Organisation recently commissioned a critical review by Sobesey and Pfaender (2002) to assess its potential for use by resource poor communities. This work identified a range of issues needing attention. Recognizing the need for further experimental evaluation and a request in the report by WHO for further data we recently undertook an experimental assessment of the H_2S test's ability to detect and quantify faecal contamination in a surficial sand aquifer used as source groundwater.

Opportunistic Value Adding CRC-WQT Pathogens Project

Evaluation of water testing methodologies is an expensive and time consuming process which can be difficult to justify in respect to developing world application where the urgent need to provide basic sanitation education and facilities takes priority. However, in 2002 when we became interested in undertaking a test comparison, some of us were also involved in an evaluation of the microbiological quality of Australian source waters using a range of pathogens and microbiological indicators (Ashbolt *et al.* 2002; Roser *et al.* 2002) on behalf of Australia's Cooperative Research Centre for Water Quality and Treatment. In Western Australia, characterization of a groundwater pollution plume/gradient was about to commence with a view to improving aquifer management and there appeared to be no impediment to concurrently undertaking H_2S testing and comparing and evaluating its performance. Indeed in hindsight, evaluation of the H_2S testing as a method for regional and remote community source water quality

assessment was seen as fully consistent with the CRCWQT project's aims and objectives, but had been overlooked due to the project being designed with the protection of large water bodies and urban populations.

Key Issues Arising from the WHO review

The Sobesey and Pfaender (2002) report has comprehensively reviewed microbiological method issues relating to the H_2S test. Key ones relating to this study are identified in Table 1. Readers are strongly recommended to examine this work not only because it relates to many aspects of this paper and details the criteria used to evaluate our findings but also because it is an excellent case study of types of issues that developers of environmental monitoring 'Appropriate Technology' should address when adapting developed world technology to different circumstances.

Not being designed with the remote site source water in mind, the CRC project could not address all of Sobesey and Pfander's (2002) concerns explicitly. However having access to this critique, provided us with the opportunity to identify and evaluate many issues raised within the limitations of the available data rather than leaving it to readers who would not have access to the primary data set.

Table 1. Methodological Limitations of Available Information on H_2S Tests

Issue	Summary/Examples of Perceived Problems
Insufficiently systematic development	Unlike other methods for faecal indicators there has been little testing of the H_2S test responses of different non coliform water bacteria on the media.
Focus on indicator bacteria	Indicator bacteria are not *per se* a primary health concern, rather it is pathogens or faecal contamination generally that are the primary concern. The H_2S test has generally been assessed for its ability to indicate the presence of indicators rather than pathogens. As faecal bacteria have their own limitations in detecting pathogens, uncertainties are compounded.
Results are empirical and correlation based	Many studies appear to be based on comparisons of mixtures of poorly characterized water samples rather than via blinded, nested and replicated experiments on well characterized samples. Inference of test reliability is based on correlation with a restricted range of analytes found in contaminated water rather than different well characterized sources and degrees of faecal or waterborne pathogen contamination.
Insufficient quantification of test responses	Most studies are based on the use of presence/absence (p/a) testing rather than quantitative measures, preventing the sensitivity of the method from being comprehensively assessed.
WHO Movement to risk based assessments	WHO is moving to a position that zero risk situations do not exist but rather that risks need to be quantified with a view to prioritization of management responses. H_2S test results *per se* have yet to be shown to be related to increased risk of disease.
Quantitative quality control data	Information on measurement accuracy and the tendency of the method to produce false positives and false negatives is limited. P/A testing poses a particular problem, as a single contaminant is sufficient to negate a test. By contrast coliform tests routinely generate numerical data which is necessarily less impacted by stray contaminants. The prospect of the test being used by non-specialists or health workers with limited technical training means that such concerns are all the more pertinent.
Variable formulations	Being produced on a study by study basis it is not clear how comparable the

	results of different studies are. Inter-laboratory comparisons frequently show that testing by commercial organizations with commercial and hence more standardized media still suffer and same can be expected with the H_2S test.
Absent in non faecally contaminated water[1] / Present in faecally contaminated water[1]	Aside from whether the method will produce H_2S in the presence and absence of its target organisms (coliform type H_2S producers) there is the problem of interference from other microorganisms and poor relationship to pathogens can potentially lead to a false positive and false negative results which cannot be accounted for by optimizing coliform detection alone.
Respond to treatment like pathogens[1] / to environmental conditions like pathogens[1] /Outnumber pathogens[1] / No Environmental multiplication[1,2]	The route of transport from primary contaminating material to consumption can be a long one in which both indicators and pathogens are subject to various stresses which will affect their abundance. The extent to which H_2S producers behave like pathogens is unclear.- This sentence is not very clear
Ease of use[1] / Cost[1]	While H_2S impregnated paper strips appear to be inexpensive, stable without refrigeration and simple to use and produce, wider issues of ease of use and cost and supporting infrastructure need assessment e.g. the cost of transporting and disposing of material; training and supervision of testers, data analysis and reporting to affected communities and efficiencies and confidence which might be gained by centralization. To take a trivial example reducing the material cost per water sample from $14 to $2 by using an H_2S assay rather than a presumptive faecal coliform count would have little effect on total costs where the total cost of acquiring and managing data only changed from $100 to $88 per sample.
Expertise/critical evaluation	Peer review and experimental designs have not been sufficiently broad. Examination of the references demonstrates review by water quality and public health experts, but little input for experts in the field of microbial media formulation or water pathogen ecology and survival.

1. Large majority of reported studies did not address issue.

MATERIALS AND METHODS

The study site was a 50 hectare urbanized zone at the north east zone of the Jandakot groundwater mound 10 km south of Perth, Western Australia (Davidson, 1995; Larsen *et al.* 1998). The primary surficial aquifer is dominated a layer of 'Bassendean Sand' 20-40 meters thick. The climate is warm temperate with daily minimum, 9oC and maximum temperatures averaging 12, 17 and 24 ^{o}C respectively (Bureau of Meterology). The urbanized study area comprised approximately 100 kennels and catteries plus owner/operator residences on properties of 0.4 hectares each. This 'kennel zone' is surrounded by native heathland and has been in operation for approximately 30 years. The zone is essentially a rhomboid oriented at 45 degrees to the compass axes. A waste management strategy is in place whereby solid animal droppings are exported off-site, but washings, liquid waste and domestic sewage are treated on site in septic tanks. Treated wastewater then leaches into the surrounding soil/sand aquifer leading to some localised groundwater nitrogen contamination (WRC 1998a; 1998b).

The hydrogeology of the Perth region has been reviewed by Davidson (1995) and the water quality of the Jandakot mound by Larsen *et al.* (1998). In the year prior to the study (2001/2002), 1.0 gigalitres was extracted in total from the 3 production bores immediately adjacent to kennel zone. With a street front of 1400 m, a surficial aquifer saturated zone depth of *ca* 20 m and aquifer void fraction of 0.4, this would induce an average horizontal movement of water of *ca* 40 m per year from the direction of the kennel

zone (i.e. from the north east). As a result the mound/kennel zone was seen to provide an opportunity to measure ability of the Bassandean sand to remove septic supernatant derived microorganisms and biomarkers over an extended time period. Water sampling was undertaken principally between September and November 2002 (i.e. Spring). Water was sampled from the following location types:

- septic tanks (5 kennel and 5 domestic; 1 occasion each; designated A01-A10);
- 'inner leachfield' zones (4 bores designated B1-B4 located 1 m horizontally to leach trench; depth to saturated zone 5.3±2.0 m; collection screens 1.35±0.17 m into the saturated zone; 3 sampling occasions);
- 'outer leachfield' zones (4 bores bores designated C1-C4 located 5 m horizontally from the leach trench; depth to saturated zone 5.3±2.0 m; collection screens 2.73 ± 0.32 m into the saturated zone; 3 sampling occasions);
- domestic bores (4 bores designated D1-D4, located 21 ± 8 m from the nearby septic tanks; collection screens reportedly 5-20 m into the saturated zone; 3 sampling occasions saturated zone X 3 occasions);
- kennel zone boundary (3 double slotted sampling bores the kennel zone western corner (E01), ; 250 m north east of the southern corner (E02) and the centre of south west boundary (E03));
- post kennel zone boundary (2 bores, one 250 m due south of southern corner (F01) and the other 100 m west of western corner (F02));
- a 'control bore' 0.7 km south-west of south west boundary .

H_2S production was assessed at Murdoch University using media prepared as described in Pillai et al. (1999) after 24, 48 and 72 hours incubation at room temperature (37 ^oC). For each sample, one 100 mL, five 20 mL, and ten 10 mL subsamples were prepared and the results used to calculate % positives responses and mpn/100 mL. MPN tables were constructed to estimate H_2S producer counts in the range 3 to 240 mpn/100 mL from the numbers of positive and negative test results obtained for each volume of a sample. Standard analytes compared were *E. coli*, enterococci, *C. perfringens*, somatic coliphage, F-specific coliphage, faecal sterol biomarkers, *Cryptosporidium* and *Giardia*, sulphite-reducing clostridia, thermotolerant coliforms, and faecal streptococci. Assay methods have been described previously (Roser *et al.* 2002) except for somatic coliphages (ISO ref) and F RNA coliphages (ISO ref).

RESULTS

General Contamination Pattern

All standard analytes showed concentration changes consistent with a contamination gradient having the following pattern: septic supernatant (A) >> exfiltration zone extracts (B & C) > contamination zone boundary (E) and remoter bores (D & F) (Table 2). H_2S testing only yielded 100% positive results for septic supernatant but all other sample types had at least some positives. Thus assessment based on a single (p/a) result was unable to distinguish unambiguously between heavily contaminated and mildly contaminated waters. However, multiple test sets, especially the ten X 10 mL arrays, provided a clear cut distinction between the most and least contaminated zones (Table 3). Comparison of geometric mean measurements for samples from within the kennel zone (A-D) (Table 2) showed a reduction in pollutant concentrations. These reductions amounting to several orders of magnitude had been expected from Schijven's (2001) observations on the removal of microorganisms by sand aquifers. While H_2S producers showed this overall pattern well, it appeared that their numbers were more comparable to those of sulphite-reducing clostridia than *E. coli* or Enterococci and that they were more sensitive to residual contamination. The test appeared to be much more sensitive than measurements of somatic and F-specific (DNA + RNA) coliphages and protozoa (*Cryptosporidium* and *Giardia*) (geometric mean[log10 standard deviation] of 14 [1.9] and 5400 [1.0] respectively). All of the latter were detected in septic supernatant samples but were completely absent from the infiltration zone samples.

Table 2 Concentrations of Selected Water Quality Parameters in Each Compartment Exposed to Septic Contamination

Parameters	Detection Limit	A. Septic Tank Super-natant	B. Inner Exfiltration Zone	C. Outer Exfiltration Zone	D. Domestic Bores	E. Septic zone Boundary	F. Post Boundary

H_2S producers (mpn 72 h)	3/100 mL	>240#	51 [1.11]	140 [0.84]	2.4 [0.30]	13 [1.0]	45 [0.96]
E. coli (cfu)	0.1/100 mL	170,000 [1.6]	1.7 [0.7]	11[1.4]	<0.1	0.2 [0.9]	0.1[0.6]
Enterococci	0.1/100 mL	15,000 [1.6]	4.2 [0.8]	4.6 [1.0]	0.2 [1.1]	0.1 [0.8]	0.8 [1.4]
SRCs	1/100 mL	155,000 [1.2]	25 [1.0]	38 [0.9]	3.8 [0.3]	0.7 [0.3]	0.9[0.6]
C. perfringens	1/100mL	6300 [1.2]	5 [1.0]	5.9 [0.7]	2.7 [0.5]	<1	<1
Somatic coliphage	1/100mL	16 [1.5]	< 1	<1	<1	<1	<1
F-specific coliphage	1/100mL	8 [1.5]	< 1	<1	<1	<1	<1
Coprostanol	1 ng/L	11,000 [1.9]	7.4 [1.2]	1.4[0.93]	1.0[0.67]	<1	<1
Cholesterol	1 ng/L	85,000 [1.0]	147 [0.50]	32[1.13]	3.5[1.2]	1.6[0.8]	0.97[0.5]
24ethyl-Coprostanol	1 ng/L	1700 [1.7]	4.9 [1.0]	2.1 [1.07]	0.57[0.17]	<1	<1
n	-	10	12	12	11	18	6

Notes:

1. Values shown are geometric means of adjusted and the standard deviation $\log_{10}$ values. Where measurements were below or above the detection limit values, statistics were calculated following substitution with half or 2X the detection limit values.
2. #Where a '<' or '>' sign is shown all samples were either greater than or less than that value.

Table 3 Comparison of % H_2S positive tests Compared with % Detection of Bacterial Indicators selected fixed detection limits.

Measurement	A. Septic Super-natant	B. Exfiltration 1 X 1 m	C. Exfiltration 2 X 5 m	D. Domestic Bores	E. Septic zone Boundary	F. External 10 m
10 mL H_2S +ve[1]	100	67/*58*	83/*73*	9/*1*	61/*33*	67/*45*
20 mL H_2S+ve[1]	100	75/*63*	92/*77*	27/*7*	44/*33*	83/*57*
100mL H_2S+ve[1]	100	67	92	18	44	67
E. coli >1 / 10 mL	100	8	42	0	0	0
E. coli >1/100 mL	100	67	75	0	5	17
Enterococci >1 / 10 mL	90	42	42	9	5	17
Enterococci >1/ 100 mL	100	75	58	9	11	50
SRCs >1/10 mL	100	75	67	0	5	17
SRCs >1/100 mL	100	92	100	91	21	17
No. of samples	10	12	12	11	18	6

Notes:

1. The first (no italicized number) is the percentage of samples in which at least 1 subsample of the volume shown was positive. The second *italicized* number is the overall average number of positive tubes for the sample group as a whole.

Remote Bore Quality and Test Specificity

The remote (domestic, boundary, post boundary) bore data at first glance was a cause for concern because the boundary and external zone areas appeared to have significant populations of H_2S producers where there were few conventional indicators, suggesting interference from non-specific H_2S producers. However, when the data from different wells were compared the explanation seemed more likely to be assay sensitivity. At the kennel zone boundary, 4 of the 5 high counts of the H_2S producers (>240 mpn/100 mL) were associated with bore E-03, which also showed the highest conventional indicator counts of the 3 boundary sites (*E. coli* and Enterococci in the range 0.4 - 44 cfu/100 mL). This bore was located in the most favorable position to intercept any contaminant plume - midway between the central production bore and the kennel zone. Similarly the elevated concentration in the 'post-boundary' zone could be accounted for by samples from bore F-02, which also had significant Enterococci numbers (4.8-56 cfu/100 mL) in all three samples compared to H_2S counts of 59, >240 and >240 mpn/100 mL. In both bores, *E. coli* and SRCs were also detected (maximum 40 and 17 cfu/100 mL respectively).

In the remaining bores sited away from the primary leach zones (E01, E02, F01, G01 and the 4 domestic bores), contamination was low. All *E. coli,* and all but 1 Enterococci, counts were < 1/100 mL, all *C. perfringens* count were < 1-10 cfu/100 mL and the maximum SRC count was 10 cfu/100 mL. This compared with relatively low H_2S counts (50% < 3 mpn/100 mL; all but one < 32 mpn/100 mL). Of a total of 30 samples only 1 with an Enterococci count of 310 cfu/100 mL could be considered a false positive.

Despite the higher average contamination levels a similar pattern was seen in the infiltration zones. The five most unequivocally contaminated samples (counts > exfiltration zone geometric mean; at least 2 indicator groups present) all had H_2S counts > 240 /100 mL. Conversely of the 24 samples analysed, 4 (all from the same household) showing no detection of H_2S producers, also had indicator counts (*E. coli* and Enterococci counts < 3 cfu/100 mL. Interestingly these samples had significant concentrations of beta stanols indicating possibly indicating disinfection having been undertaken.

Overall these data indicated that the H_2S test was detecting localized groundwater contamination probably from plumes of material transported from the kennel zone septics. Quantitation using the mpn estimates was preferable to simple p/a testing in that it allowed interpretation of variations in contaminant levels. While the identities of the H_2S producers could not be determined the majority were not members of the 3 bacterial indicator groups, as the mpn counts generally far exceeded those of the presumptive and confirmed indicator counts (Table 2).

False Positives and Negatives

'False positive' H_2S test results were much more likely than 'false negatives. However this begged the question of what was a 'false positive' when aquifer water quality seemed satisfactorily assessed and there were multiple possible reference analytes. If the criterion was H_2S positive in the absence of *E. coli* then there were many. On the other hand *E. coli* enumeration generated many false negatives with respect to Enterococci and SRCs. Previously it had been found (Roser *et al.* 2002) that Enterococci were preferable as a contaminant indicator to *E. coli* in the Perth aquifers. Table 4 shows a comparison of the performance of *E. coli* and the basic p/a H_2S test using Enterococci as the reference standard. As with Table 2 it can be seen that the Enterococci assay tended to yield results midway between H_2S and *E. coli* assays despite its use of lower detection limit than the H_2S test.

Another observation relevant to evaluating the H_2S test was the occurrence of 'false positive' and 'false negative'-like results arising from the standard operating procedures and normal constraints of the commercial laboratory. In hindsight not all appropriate sample volumes were assayed. Confirmed *C. perfringens* proved hard to detect due to swarming of 'contaminant' - presumably other soil Clostridia present in groundwater. Only restricted numbers of SRCs could be confirmed probably leading to reporting of zero *E. coli* and *C. perfringens* counts on several occasions when this might not have been the case. Somatic coliphage, F-specific coliphage and protozoan parasites though all detected in significant numbers in the primary septic supernatant were of little value as passive measures of contamination as none were detected within the aquifer itself. Sterols though arguably the most general indicators of contamination, as they comprise 1% of human faecal dry matter were useful but are currently too expensive for routine assessments. Overall these issues relating to faecal contamination analysis do not technically generate 'false negatives', but their impact on assaying water quality management is potentially similar and underline the need to evaluate not only statistics on the H_2S test but place it into a context which recognizes the limitations in conventional microbiological assays.

Table 4 Comparison of H_2S and *E. coli* test performance with Enterococci counts of Aquifer samples

Comparison Criterion	**H_2S (p/a in 100 mL)**	***E. coli***
No. of tests: total/positives/negatives	64/34/30	64/17/47
No. of false negatives (Enterococci count range)	4 (0.1 - 6)	13 (0.3-310)
No. of false positives (*range of E. coli counts associated with false*	13	5 (*0.1-390*)

positives)		
No. of false positives with no other indicator detected	4	1

Notes
1. Measurements used in comparison were bacterial indicators in all samples other than those from the septic tanks. H_2S p/a is based on the results of the 72 h readings of each 100 mL sample.

DISCUSSION

While Sobsey and Pfaenders' (2002) concerns were justified, the H_2S test performance can be improved by the adoption of a multiple tube or mpn approach and appropriate monitoring program design (e.g. protection of source water and identification of contamination). For aquifers which can have a strong to variable filtration effect it may be a very valuable survey technique. The evaluation undertaken here was superior to others in the literature as the system assessed was not contrived, was geographically well defined, and involved replicated testing of a diversity of indicators pathogens and biomarkers to assay samples from locations differing markedly in the extent of contamination but linked hydrologically in space and time. The major limitation was that the evaluation of the H_2S was opportunistic and constrained to comparative response studies e.g. no data on specific hydrogen sulphide producing populations was collected. However being a well-define 'natural experiment' in a hydrological steady state the potential exists for addressing such issues in the future through a cross-disciplinary study.

Evaluation against key WHO Report Concerns

The study could not fully evaluate all the WHO Report concerns, however, aspects of most have been addressed in respect to a model aquifer supply.

- **Extent to which measurements are indicative of faecal contamination/Focus on indicator bacteria / Results are empirical and correlation based**: While indicator bacteria were a major component of the study, comparisons focused on the best defined and/or persistent indicators (*E. coli*, Enterococci, *C. perfringens*) available and complemented this with other assays data. While interpretations were still correlation based, the range of supporting information made them more reliable. Detailed definition of the study system plus the current database necessarily sets up testable hypotheses and regarding aquifer water quality that could be subject to further studies.
- **Insufficiently systematic development**: A study system was identified and systematically characterized with application of pathogens, indicator and H_2S testing to groundwater protection in mind. Earlier studies (e.g. Genthe & Frank, 1999), have surveyed diverse source waters whose individual characteristics are not well described making comparison difficult. Our study complements such work by focusing on a single well defined and characterized source water.
- **Insufficient quantitation of test responses**: This was addressed by the use of an mpn approach and studying a range of contamination levels. In practice the sensitivity of the test limited the dynamic range of mpn measurements of aquifer samples but this could be addressed by testing smaller volume sub-samples as well (e.g. 1 mL volumes as part of a 3x3 mpn test array). The sensitivity of the assay suggests that total assay volumes < 100 mL could yield useful data and lead to savings in materials and transport costs.
- **WHO movement to risk based assessments**: The main CRC-WQT study was concerned at evaluating the risk posed by septic entry and surveys were designed accordingly. The H_2S testing system appears capable of detecting the presence of pollutant plumes in sandy aquifers and hence being a tool in on ground risk assessment i.e. a Sanitary Survey.
- **Quantitative quality control data**: Controls (blanks; replicates and spikes) were assayed as part of the CRC-WQT project. Thus there was confidence in data on the quality of water in the aquifer.
- **Absent in non-faecally contaminated water/ Present in faecally contaminated water**: The likely locations of contaminated and clean water were identified from a knowledge of the hydrogeology and past water quality surveys of the site and confirmed by conventional tests.
- **Respond to treatment like pathogens/Respond to environmental conditions like pathogens:** The Jandakot aquifer appears to behave as a natural sand-filter with bacterial size or larger particles being effectively removed over the scale studied. The detection of H_2S producers in the leach zones suggest the test may be useful for monitoring bacterial removal by sand filters.

- **Outnumber pathogens/No Environmental multiplication/Detects non-pathogenic bacteria**: H_2S producers outnumbered the protozoa and the virus models (coliphages), and the conventional indicators tested.
- **Ease of use/Cost**: No logistics difficulty was encountered. Full costing was not undertaken but the cost of test materials for 80 samples X 16 bottles was A$1000.
- **Expertise/critical evaluation**: The study team included experts experienced in sample collection, research, commercial testing, and the H_2S test. The WHO report provided evaluation criteria.
- **Variable formulations/Organisms actually measured**: not addressed

Use of the H_2S test in other situations

Given the sensitivity of the assay and the significant numbers of indicators present in surface source waters (Ashbolt *et al.* 2002); presence/absence testing would be of little value in the latter case. MPN testing might provide useful information and preliminary assessments might be undertaken by water testing authorities as an add on to their routine monitoring. Most relevant would be the assessment of riparian extraction wells impacted during storms or floods. Application to other aquifer types needs to be tested. The system studied here has been heavily leached and measurements of cholesterol and sitosterol indicate that little organic matter is permeating the deeper strata. Depending on the biological productivity of the surface ecosystem and aquifer permeability this might not always be the case leading to the development of H_2S producing bacterial communities. Testing of sand filter efficiency might be a further application.

An examination of Standard Methods (ref) will show that both *E. coli* and Enterococci assays are most suited to rapid processing (4-6 hours), the availability of sample coolant (4 ^oC), stable *ca* body temperature incubation. This reflects not only analyte instability but also the circumstances of the test's origins (e.g. urban societies in temperate climates with good transport systems, reliable power, sufficient technical specialists, revenue streams, monitoring of the quality of nearby source waters). The testing systems existing now are efficient and effective but are only possible because of this extensive supporting infrastructure. As soon as these conditions are not met, problems and adaptation occur. This illustrated in Australia where distances between rural and urban communities are much greater than in Europe. The result is a more liberal sample national transport standard (2-12^oC within 24 hours) (IDEXX). And in extreme situations, routine testing of remote community water supplies is often not even undertaken (Nair *et al.* submitted for publication).

The reasons for promoting coliforms as a standard are good ones e.g. their *de facto* place as a global water quality standard for prioritizing water management activities and resource allocation. However, where the test becomes completely unworkable for a range of logistical, financial and human resource reasons, alternatives must be sought. The design and areas of use of the H_2S method reflects and addresses such logistics and resource problems as well as the traditional focus on coliform indicators. Conversely the limitations of indicators discussed elsewhere (Sobesey and Pfander 2002) and illustrated in this study by their poorer survival compared to more persistent indicators in particular Enterococci and *C. perfringens*.

CONCLUSIONS

The move to a risk based approach to water quality management tacitly promotes the introduction of alternative water quality testing approaches(or more radical adaptation of existing ones) which are judged not only by technical criteria but also by logistical and resource ones as the primary criterion becomes public health status and its protection. Looked at from this point of view the H_2S test may be seen rather than a 'poor persons coliform' test as a distinct assay in its own right which has been developed to address real public health needs. The Colisure and Colilert systems (ref) are solutions to related problems they address (at least in western countries) logistics issue. But others approaches are possible. For example *C. perfringens* and somatic coliphages are recognized as possibly secondary faecal indicators. While fuller investigations need to be undertaken, their basic biology indicates that samples should be much more stable and less impacted by the instability problems of *E. coli* and Enterococci. Alternatively techniques of transport/enrichment media long used in hospital environments might be adapted to the maintenance of indicators over periods > 24 hours. Surrogate measures needs further exploration. Nitrate testing may be a useful surrogate for identifying groundwater contamination. Finally there is the simple

expedient of physically examining a water source for high risk features such as coarse substrate and source protection arrangements which can be as effective in microbial control as indicator assays.

One danger involved in innovation is that standardization and quality of assessments is lost. This is an implicit concern in the WHO review. The H_2S testing situation provides an opportunity for systematically developing protocols for introducing new technology which balance the need for scientific rigor with access of target communities to the benefits such technology might bring.

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REFERENCES

Ashbolt et al. 2002

ColiSure and Colilert references

Davidson, W. A. (1995) Hydrogeology and Groundwater Resources of the Perth Region Western Australia. Geological Survey of Western Australia Bulletin 142, Perth. ISBN 0-7309-6502-3.

Genthe, B. and M. Franck (1999) A tool for Assessing Microbial Quality in Small Community Water Supplies: An H2S Strip Test. WRC Report No. 961/1/99, 33 pages. Water Research Commission, Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, South Africa.

ISO coliphage methods

Larsen, R. M., Watkins, K. L., Steel, N. A., Appleyard, S. J. and Bauld, J. (1998). A Groundwater Quality Assessment of the Jandakot Mound Swan Coastal Plain Western Australia. AGSO Record 1998/18, Canberra.

J. Nair, K. Mathew and G.E. Ho (2004) Experiences with Implementing the H2S method for Testing Bacterial Quality of Drinking Water in Remote Aboriginal Communities in Australia. submitted to Journal for Water and Health

Pillai J., .Mathew K., Gibbs R. & Ho G.E. 1999 H2S Paper Strip Method-A Bacteriological Test for Faecal Coliforms in Drinking Water at Various Temperatures. Water Science and Technology. 40(2): 85-90.

Roser et al 2002

Jack F. Schijven Virus Removal from Groundwater by Soil Passage Modeling, Field and Laboratory Experiments 2001 te 1 ISBN 90-646-4046-7 Printed by Ponsen & Looijen B.V., Wageningen

Sobsey M. D. and Pfaender F. K. (2002). Evaluation of the H2S Method for Detection of Fecal Contamination of Drinking Water. Report WHO/SDE/WSH/02.08. Water, Sanitation and Health Department of Protection and the Human Environment World Health Organization Geneva.

Waters and Rivers Commission, (1998a). Water Quality Protection Guidelines No. 25 Waste Management of kennel operations with the Jandakot UWPCA. Perth ISBN 0-7309-7332-8.

Water and Rivers Commission, (1998b). Technical Report on Kennel Waste Disposal and Management for Public Drinking Water Source Areas. WRP 29, Perth .

Water Corporation, (1998). Metropolitan Production Bores Water Quality Data 1996/1997. Report WDQ 01/98, Perth.

VBM Oceanics (2003) HLN OSRAS Working Paper: Logic Matrices Workshop.

Standard Methods