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ORIGINAL ARTICLE

Usefulness of the hydrogen sulfide test for assessment of water quality in Bangladesh

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Abstract

Aim: To evaluate the usefulness of the hydrogen sulfide (H_2S) test for assessing water quality in Bangladesh.

Methods and Results: We tested 382 water samples from a variety of sources using locally produced H_2S test kits and laboratory-based membrane filtration for the detection of *Escherichia coli*. Compared with membrane filtration, H_2S tests, when incubated for 24 h, had both a sensitivity and positive predictive value (PPV) of <40% when analysis was restricted to water samples with *E. coli* levels below 100 colony forming units (CFU) per 100 ml. In contrast, for *E. coli* levels from 1000 to 9999 CFU per 100 ml, sensitivity was 94% and PPV 88%; specificity was 97% and negative predictive value was 99%.

Conclusions: The hydrogen sulfide test, when incubated at 24 h, is a promising alternative for assessing water quality where *E. coli* levels may be high. An improved understanding of the incremental impact of contamination level on health is needed to better determine its usefulness.

Significance and Impact of the Study: The hydrogen sulfide test is inexpensive, easy to use and portable. Its use may allow rapid assessment of water quality in situations where cost or logistics prevent use of other testing methods, such as in remote areas or during floods and other natural disasters.

Introduction

Diarrhoeal disease is a major killer of Bangladeshi children, causing an estimated 23 000 child deaths annually (National Institute of Population Research and Training 2004; UNICEF 2006). During Bangladesh's annual rainy season from June to September, widespread flooding predictably amplifies microbial contamination of surface water, and increases the risk of diarrhoeal disease (Faruque *et al.* 2005). Although most of Bangladesh's 142 million inhabitants (World Bank 2006) have access to tubewell water, 46% of tubewells contain levels of arsenic exceeding the WHO guideline value of 10 μg l^{-1} (BGS/DPHE 2001), placing a large population at risk of life-threatening complications of arsenic toxicity (Smith *et al.* 2000). As a result, the National Policy for Arsenic Mitigation (Government of Bangladesh 2004) recommends using surface water for drinking, treated either at the point of collection or at the household level.

Surface water treatment programmes advocated as part of arsenic mitigation strategies should include water quality monitoring to evaluate the effectiveness of treatment and risk to the consumer, as surface water is frequently highly contaminated. Testing water for indicators of faecal contamination is used as a proxy for diarrhoeal disease risk. Currently available water quality tests are less expensive and less time-consuming than diarrhoeal disease surveillance, but they are still costly and logistically difficult to use and interpret.

The most established method for testing water quality is the identification of *Escherichia coli*, an indicator of faecal contamination, using a standard membrane filtration protocol (World Health Organization 2004). Samples must be collected aseptically and carefully transported on ice to an adequately equipped laboratory, where this expensive ($7·40 per test in Bangladesh) and time-consuming process is carried out by skilled microbiologists. Because of these limitations, this method becomes impractical for regular field use, especially in difficult environments, such as during floods. Qualitative or semi-quantitative alternatives to standard membrane filtration that use ultraviolet fluorescence for detection of *E. coli* are still expensive and require incubation at a set temperature for at least 24 h.

One alternative to these tests is the hydrogen sulfide (H_2S) presence/absence test, which was developed as an inexpensive field-based water quality test in the early 1980s (Manja *et al.* 1982). A sample of water is added to a bottle which contains reagents including ferrous iron and sulfate salts, and the sample is observed for colour change over 24–48 h. The H_2S test rests on the observation that enteric bacteria reduce sulfur to hydrogen sulfide, which has a characteristic strong odour and forms a black iron sulfide precipitate in the presence of ferrous iron. In Bangladesh, UNICEF supported the introduction of the hydrogen sulfide test, and the NGO Forum for Drinking Water Supply & Sanitation (NGO Forum), a Dhaka-based consortium of nongovernmental organizations and community-based organizations, now produces an H_2S vial for water quality testing at a cost of $0·20 per test.

Although the H_2S test has been used globally for more than two decades, it has never been standardized, rigorously evaluated or correlated with disease risk. A recent comprehensive review of the H_2S test described numerous evaluations of its performance, and concluded that until more comprehensive and rigorous studies are performed, the H_2S test could not be widely recommended for testing faecal contamination of drinking water (Sobsey and Pfaender 2002). H_2S testing was recommended with caution in specific situations: where other water quality tests are not available and for educational or motivational purposes.

In a recent evaluation of the H_2S test against established indicators of faecal contamination, a semi-quantitative, most probable number (MPN) H_2S test method was used, and this method was found to be effective in distinguishing between water sources with different levels of faecal contamination (Roser *et al.* 2005).

To our knowledge the H_2S test has never been evaluated in Bangladesh, where standard membrane filtration tests are often not practical. To establish its performance in Bangladesh, we evaluated the usefulness of the H_2S test as an inexpensive, field-based alternative for assessing water quality.

Materials and methods

We obtained water samples from a variety of sources from different regions of Bangladesh: dug wells, tubewells, surface water, rainwater from rainwater collection tanks and water stored by household members in typical containers. In addition, we obtained samples of water treated by the chulli water purifier, a locally invented household water treatment device that combines sand filtration and heat pasteurization (Islam and Johnston 2006). Chulli water purifier sand-filtered samples, representing incompletely treated water samples, and chulli water purifier sand-filtered, heat-treated samples, were both included.

A variety of sources were sampled in order to test water from different regions of the country with different microbiological and chemical characteristics. At least 500 ml of water was collected using aseptic technique. Twenty millilitres was inoculated into a hydrogen sulfide test vial in the field and transported to the laboratory at ambient temperature; the remainder of the sample was placed on ice and taken to the laboratory for further testing. All laboratory analysis was done at the Environmental Microbiology Laboratory of ICDDR,B in Dhaka, Bangladesh.

Hydrogen sulfide tests

The NGO Forum supplied H_2S test vials for use in this evaluation. The medium was formulated by combining 20·0 g peptone, 1·5 g dipotassium hydrogen phosphate, 0·75 g ferric ammonium citrate, 1·0 g sodium thiosulfate and 1·0 g bile salt No. 3 and suspending in distilled water to 50 ml volume. One millilitre of this solution was added to a cotton ball and inserted into a glass vial. Bile salts were added to preferentially inhibit the growth of nonenteric sulfur-reducing bacteria. The presence of sulfur-reducing bacteria, or certain abiotic chemical reactions, causes iron sulfide to precipitate, turning the water to black. The kits use a flattened vial with a screw top cap and a plastic inner cap (Fig. 1). All components were sterilized by autoclaving. Vials were produced by NGO Forum at two different times during the study period.

During inoculation of the H_2S vials, the plastic inner cap was removed and replaced as aseptically as possible. Hydrogen sulfide vials were vigorously shaken immediately after inoculation to inhibit the growth of anaerobic and microaerophilic organisms. Inoculated vials were kept at room temperature (25°C) and read for the presence or absence of colour change (clear to black) at 1, 24, 48 and

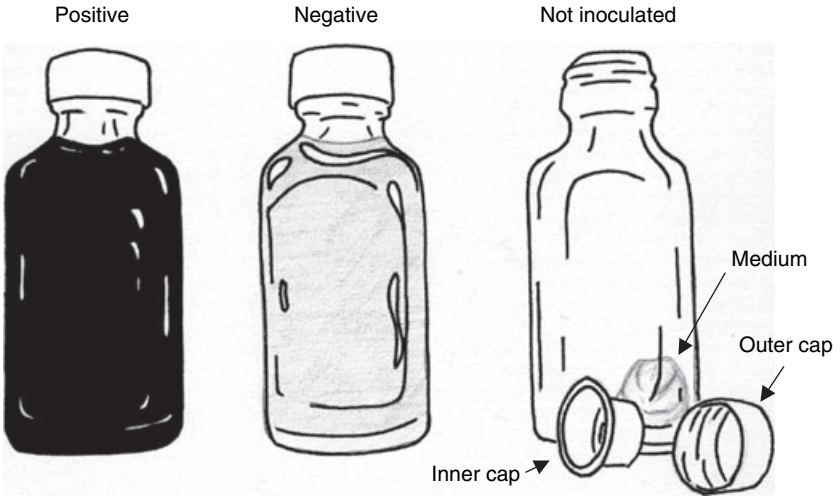

Figure 1 Hydrogen sulfide vial produced by NGO Forum, Bangladesh.

72 h. For each day of testing, one negative control test was also performed using sterile water. The test was read as positive if the colour changed from clear to black; as the colour change was easy to detect, no intermediate category was used. A 1 h reading was performed to check for early positives, indicative of chemical induced sulfide formation, rather than growth of sulfur-reducing bacteria (Sobsey and Pfaender 2002).

Enumeration of *E. coli*

In the laboratory, water from the original 500 ml or 1 l plastic water sample bottle was used to enumerate *E. coli* by the membrane filtration method. Modified membrane-thermotolerant *E. coli* agar (Benton Dickinson, Sparks, MD, USA) was prepared and the membrane filtration test was carried out following the procedures described by the United States Environmental Protection Agency (2002). An aliquot of 100 ml of water was filtered through the Millipore membrane filters; filter papers were then placed on modified membrane-thermotolerant *E. coli* agar media and incubated at 35°C for 2 h and then at 44·5°C for another 22 h. Red or magenta colonies were then counted. The presence of ≥1 *E. coli* colony forming unit (CFU) per 100 ml was defined as a positive *E. coli* test. Positive water samples were categorized into low, medium, high, very high and exceedingly high-level contamination groups (1–9, 10–99, 100–999, 1000–9999 and ≥10 000 CFU per 100 ml).

We calculated sensitivity, specificity, overall agreement, positive predictive value (PPV) and negative predictive value (NPV) along with the corresponding 95% exact confidence intervals (CI), for the H_2S test read at 24, 48 and 72 h compared to the identification and enumeration of *E. coli* by membrane filtration. Overall agreement was determined by calculating the sum of samples that were found positive by both tests or negative by both tests and dividing that sum by the total number of tests performed. The membrane filtration test was used as the standard by which to evaluate the H_2S test.

Results

A total of 382 samples from seven different types of sources were collected. One negative control H_2S test was performed on each of the six different days of water sample collection during this study; all six were negative at 72 h. No H_2S tests were positive at 1 h, indicating that all samples were free of sulfide and sulfur-reducing compounds. Seven samples (two from stored drinking water, two from chulli water purifier heat-treated water and three from tubewells) with negative *E. coli* results yielded positive H_2S results when read at 24 h.

Comparisons of tests

When H_2S tests were compared to identification of any *E. coli* by standard membrane filtration, sensitivity increased with incubation time from 69% (95% exact CI 62–76) at 24 h to 94% (95% exact CI 89–97) at 72 h, while specificity showed the opposite trend (Table 1), decreasing from 97% (95% exact CI 93–99) at 24 h to 58% (95% exact CI 51–65) at 72 h.

The relationship between hydrogen sulfide tests and quantitative *E. coli* results was then examined (Fig. 2). The sensitivity of H_2S testing increased with the level of *E. coli* contamination, increasing from 7% (95% exact CI 0–32) to 94% (95% exact CI 84–99), for low- and very-high-level contamination groups, respectively, when incubated for 24 h. This trend was statistically significant for H_2S tests

Table 1 Sensitivity, by level of *Escherichia coli* contamination, and specificity of H_2S test, Bangladesh, 2005

Duration of H_2S test incubation (h)	*E. coli* level of water samples (CFU per 100 ml)	Number of samples in category	Sensitivity, % (95% exact CI)	Specificity, % (95% exact CI)
24	<1	219		97 (93–99)
	1–9	15	7 (0–32)	
	10–99	15	27 (8–55)	
	100–999	40	53 (36–68)	
	1000–9999	52	94 (84–99)	
	≥10 000	41	93 (80–98)	
	All ≥1	163	69 (62–76)	
48	<1	219		80 (74–85)
	1–9	15	27 (8–55)	
	10–99	15	73 (45–92)	
	100–999	40	90 (76–97)	
	1000–9999	52	100 (93–100)	
	≥10 000	41	100 (91–100)	
	All ≥1	163	88 (82–93)	
72	<1	219		58 (51–65)
	1–9	15	60 (33–84)	
	10–99	15	93 (68–100)	
	100–999	40	93 (80–98)	
	1000–9999	52	100 (93–100)	
	≥10 000	41	100 (91–100)	
	All ≥1	163	94 (89–97)	

read at 24, 48 or 72 h ($P < 0{\cdot}01$ for each of the three incubation periods, Cochran–Armitage test for trend).

H_2S tests that required a longer incubation time to turn positive had a lower intensity of *E. coli* contamination. For the 120 water samples that were positive by hydrogen sulfide testing at 24 h, the median *E. coli* count was 3000 CFU per 100 ml (mean, 13 891). For the 68 water samples that subsequently turned positive at 48 h, the median *E. coli* count was <1 CFU per 100 ml (mean, 695), a statistically significant difference ($P < 0{\cdot}01$, Wilcoxon's two-sample rank sum test).

The overall agreement between the presence or absence of *E. coli* by membrane filtration and H_2S results was 85% (95% exact CI 81–88) when the H_2S test was read at 24 h, 84% (95% exact CI 79–87) at 48 h and 73% (95% exact CI 69–78) at 72 h.

For the limited to low or medium contamination groups, PPV was below 40% regardless of H_2S test incubation time (Table 2). For the high, very high and exceedingly high contamination groups, PPV was 75% (95% exact CI 55–89), 88% (95% exact CI 76–95) and 84% (95% exact CI 71–94), respectively, but was consistently below 75% for longer incubation periods, as the number of false-positive tests increased. NPVs were greater than 80% for each incubation period and *E. coli* contamination level (Table 2).

Test characteristics for water sources

The intensity of *E. coli* contamination, the frequency of *E. coli* contamination and the frequency of positive H_2S results showed similar patterns for different water sources. Chulli water purifier heat-treated water and tubewell water both had a median of <1 CFU per 100 ml *E. coli*, in contrast to 6000 CFU per 100 ml *E. coli* for surface water (Fig. 3). Tubewell (3%) and heat-treated water (27%) were infrequently positive by H_2S testing read at 24 h, followed by stored drinking water (52%), dugwell water (54%) and rainwater (64%), and finally sand-filtered water (92%) and surface water (94%); this pattern was also similar for *E. coli* tests (Fig. 4).

Similarly, sensitivity of H_2S testing with 24 h incubation was only 15% (95% exact CI 4–34) for tubewells, a water source with infrequent and low intensity *E. coli* contamination, compared to 94% (95% exact CI 83–99) for surface water, a water source with frequent and high intensity *E. coli* contamination.

Discussion

When incubated for 24 h, the hydrogen sulfide test used in Bangladesh has high PPV and NPV for highly contaminated water samples, and is a promising alternative for assessing water quality under field conditions. The test is inexpensive, portable, simple to use and there was no evidence of contamination due to field use.

In this study, the hydrogen sulfide test was compared to testing for *E. coli*, a commonly accepted but still imperfect proxy for diarrhoeal disease risk. The high level of overall agreement found between the hydrogen sulfide and *E. coli* tests suggests that both tests measure similar or highly related characteristics.

The lack of data regarding diarrhoeal disease risk with low-level *E. coli* contamination limits the ability to interpret the usefulness of the H_2S test for relatively clean water sources, such as tubewells and heat-treated water. One study from the Philippines demonstrated higher rates of diarrhoea among children with >1000 CFU per 100 ml of *E. coli* in drinking water sources (Moe *et al.* 1991). These patterns may vary from region to region, depending on the local microbial ecology and its interaction with the local population. If a similar association exists in Bangladesh, the H_2S test may be extremely useful because it performs best with water samples with >99 CFU per 100 ml of *E. coli*.

Evidence of substantial quantities of sulfur-reducing chemicals or sulfide, which would cause early positive reactions, was not found. Besides measuring the level of *E. coli* contamination, we did not evaluate other characteristics of water that may affect test performance.

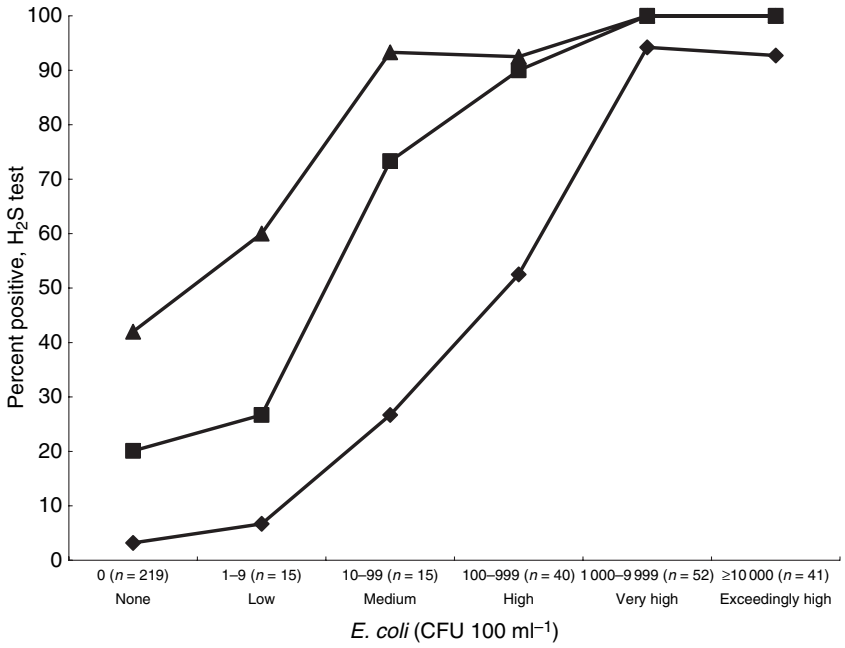

Figure 2 Proportion of positive H_2S tests by level of *Escherichia coli* contamination (n = 382). (–◆–) 24 h; (–■–) 48 h and (–▲–) 72 h.

Table 2 Positive (PPV) and negative predictive values (NPV) of H_2S test, for different *Escherichia coli* contamination levels, Bangladesh, 2005

Duration of H_2S test incubation (h)	*E. coli* levels of water samples included in analysis (CFU per 100 ml)	Number of H_2S positive samples	PPV, % (95% exact CI)	Number of H_2S negative samples	NPV, % (95% exact CI)
24	<1, 1–9	8	13 (0–53)	226	94 (90–97)
	<1, 10–99	11	36 (11–69)	223	95 (91–98)
	<1, 100–999	28	75 (55–89)	230	92 (87–95)
	<1, 1000–9999	56	88 (76–95)	214	99 (96–100)
	<1, ≥10 000	45	84 (71–94)	214	99 (96–100)
	≥0	120	94 (88–98)	261	81 (76–85)
48	<1, 1–9	48	8 (2–20)	186	94 (90–97)
	<1, 10–99	55	20 (10–33)	179	98 (94–99)
	<1, 100–999	80	45 (34–57)	179	98 (94–99)
	<1, 1000–9999	96	54 (44–64)	175	100 (98–100)
	<1, ≥10 000	85	48 (37–59)	175	100 (98–100)
	≥0	188	77 (70–82)	194	90 (85–94)
72	<1, 1–9	101	9 (4–16)	133	95 (90–98)
	<1, 10–99	106	13 (7–21)	128	99 (96–100)
	<1, 100–999	129	29 (21–37)	130	98 (93–100)
	<1, 1000–9999	144	36 (28–45)	127	100 (97–100)
	<1, ≥10 000	133	31 (23–39)	127	100 (97–100)
	≥0	245	62 (56–69)	137	93 (87–96)

As the H_2S test is nonstandardized, the results that we found are only strictly valid for the specific test kit that we used. Other formulations of the H_2S test with a different bottle type, material preparation, medium composition, sample volume, incubation time or incubation temperature may perform differently.

The use of bile salts in the test formulation may have been helpful in preventing the growth of sulfur-producing

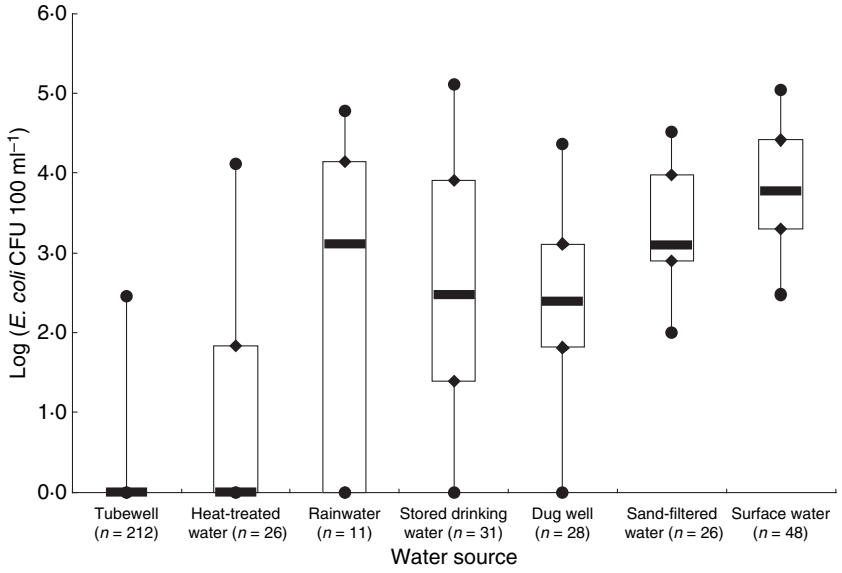

Figure 3 Quantitative *Escherichia coli* results by water source, by quartile (*n* = 382). (●) Minimum and maximum; (◆) quartile 1 and 3; (▬) median.

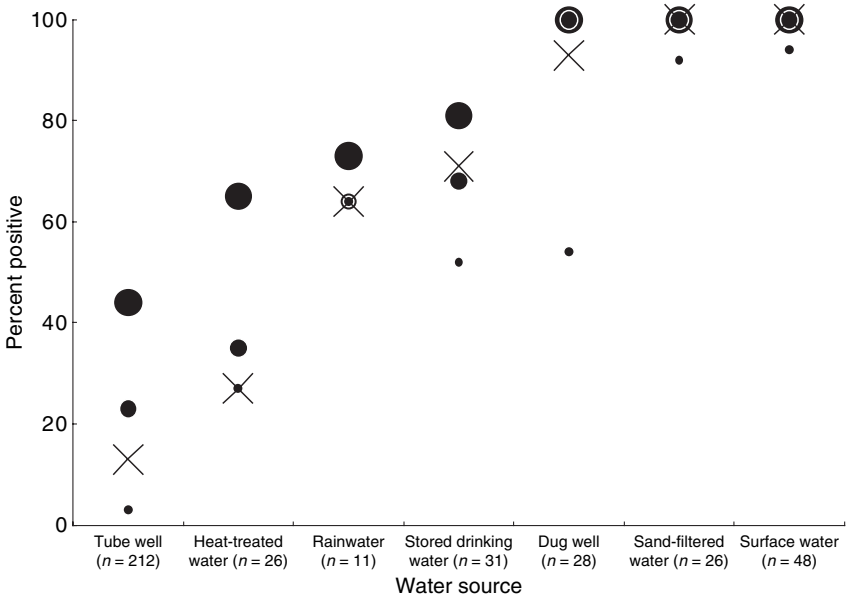

Figure 4 Proportion of water samples that tested positive for contamination by water source and type of test (*n* = 382). (⬤) H_2S at 72 h; (●) H_2S at 48 h; (•) H_2S at 24 h and (×) *E. coli*.

nonenteric bacteria, but could have also decreased sensitivity and NPV by decreasing the detection of *Clostridium perfringens* (Sobsey and Pfaender 2002).

This study was performed by incubating vials at room temperature (25°C) in the ICDDR,B laboratory, which may be slightly cooler than the ambient temperature in Bangladesh during most of the year. Warmer temperatures of 30–35°C would be expected to both increase the growth of bacteria and inhibit the growth of nonenteric bacteria (Sobsey and Pfaender 2002), and could possibly cause all measures of test accuracy to improve. However, this would need to be confirmed through further investigation.

The NGO Forum can currently produce 100 H_2S kits per day, and this capacity can be increased if H_2S kits are used in Bangladesh. The inner plastic cap was clumsy to remove; specificity might be further improved with a cap

that retains sterility and prevents leakage, but does not include an inner cap, such as a single cap with a rubber cushion. Rigorous quality control is essential, both to achieve proper initial sterilization of vials, and to ensure that vials can be opened, closed and transported without introducing contamination.

Because of the low number of samples obtained from some water sources, we were unable to determine the effect of water source on test performance independent of level of contamination with *E. coli*. Some characteristics of different water sources may theoretically affect performance of the H_2S test; for example, water from dug wells or tubewells may contain sulfur-reducing deep soil bacteria, which may cause false-positive results. However, we did not identify a substantial independent effect of water source, suggesting that if there is a water source specific effect on test performance, it is a less important determinant of test performance than *E. coli* contamination.

Also, in the seven water samples that were H_2S test positive but negative for *E. coli*, we did not attempt to identify specific bacterial species present. This information would be helpful in determining if the membrane filtration test failed to identify faecal organisms, or if the organisms causing the hydrogen sulfide tests to turn positive were sulfur-reducing nonenteric bacteria.

Studies in Bangladesh on the relationship of level of contamination to risk of diarrhoeal illness are critical to better understand what incremental effect eliminating enteric organisms in water would have on health, and the relative usefulness of water quality tests which differ in their ability to detect low-level contamination. Studies of water quality interventions, especially those of the health impact of such interventions, should include both hydrogen sulfide testing as well as testing for *E. coli* to examine their respective associations with illness. The usefulness and additional cost of the MPN method should also be considered in future evaluations of the H_2S test in Bangladesh. Finally, efforts should be made to standardize the formulation and testing protocol for H_2S tests to allow comparison across regions.

Based on this study, use of hydrogen sulfide testing with a 24-h incubation period is recommended with caution and only in situations where it is too expensive or impractical to use more established water quality testing methods, such as during the annual floods or in remote areas in Bangladesh. As with all water quality tests, results should be interpreted within the larger context of drinking water risk assessment tools (World Health Organization 2004).

Acknowledgements

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References

BGS/DPHE (2001) *Arsenic Contamination of Groundwater in Bangladesh* ed. Kinniburgh, D.G. and Smedley, P.L., Vol. 2. Final Report, BGS Technical Report WC/00/19. Keyworth, UK: British Geological Survey. Available at: http://www.bgs.ac.uk/arsenic/bangladesh/reports.htm [accessed 5 November 2006].

Faruque, S.M., Naser, I.B., Islam, M.J., Faruque, A.S.G., Ghosh, A.N., Nair, B., Sack, D.A. and Mekalanos, J.J. (2005) Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proc Natl Acad Sci USA* **102**, 1702–1707.

Government of Bangladesh (2004) *National Policy for Arsenic Mitigation*. Dhaka: Government of Bangladesh.

Islam, M.F. and Johnston, R.B. (2006). Household pasteurization of drinking-water: the chulli water-treatment system. *J Health Popul Nutr* **24**, 356–362.

Manja, K.S., Maurya, M.S. and Rao, K.M. (1982) A simple field test for the detection of fecal pollution in drinking water. *Bull WHO* **60**, 797–801.

Moe, C.L., Sobsey, M.D., Samsa, G.P. and Mesolo, V. (1991) Bacterial indicators of risk of diarrhoeal disease from drinking-water in the Philippines. *Bull WHO* **69**, 305–317.

National Institute of Population Research and Training (2004) *Bangladesh Demographic and Health Survey (BDHS) 2004 (Preliminary Report)*. Dhaka, Bangladesh: Mitra and Associates and Macro International Inc.

Roser, D.J., Ashbolt, N., Ho, G., Mathew, K., Nair, J., Ryken-Rapp, D. and Toze, S. (2005) Hydrogen sulphide production tests and the detection of groundwater faecal contamination by septic seepage. *Water Sci Technol* **51**, 291–300.

Smith, A.H., Lingas, E.O. and Rahman, M. (2000) Contamination of drinking water by arsenic in Bangladesh: a public health emergency. *Bull WHO* **78**, 1093–1103.

Sobsey, M.D. and Pfaender, F.K. (2002) *Evaluation of the H2S Method for Detection of Fecal Contamination of Drinking Water*. Geneva, Switzerland: World Health Organization.

UNICEF (2006) *Bangladesh Health Statistics*. Available at http://www.unicef.org/infobycountry/bangladesh_bangladesh_statistics.html [accessed 16 May 2006].

United States Environmental Protection Agency (2002) *Method 1603: Escherichia coli in Water by Membrane Filtration Using Modified Membrane-thermotolerant E. coli Agar (Modified mTEC)*. Washington, DC: Government Printing Office.

World Bank (2006) *World Development Report 2007*. Washington, DC: World Bank.

World Health Organization (2004) *Guidelines for Drinking Water Quality*, 3rd edn. Geneva, Switzerland: World Health Organization.

