

# Effectiveness of H<sub>2</sub>S strips for predicting bacterial contamination in water

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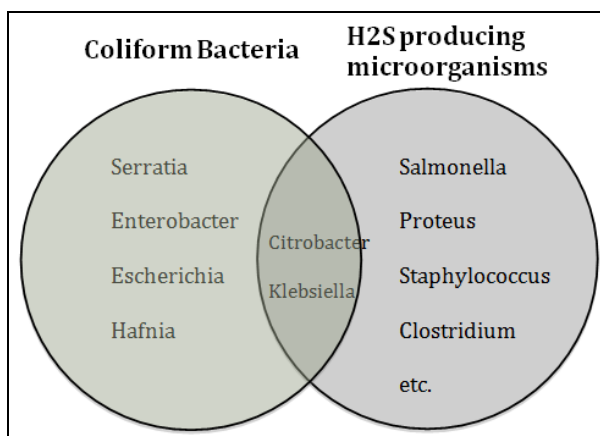
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## I. Brief Overview

Hydrogen sulfide (H<sub>2</sub>S) strips are simple and inexpensive tests for predicting bacteriological contamination in water sources. While not as accurate as some laboratory tests (e.g., Membrane filtration), they provide reasonably consistent results on whether or not water sources are contaminated with fecal material. Most commonly, the H<sub>2</sub>S strip is used as a presence/absence test for community-based surveillance (UNICEF, 2008). For example, the test can be used to empower communities with direct knowledge of the health of their water supplies—allowing them to demand improvements or take remedial actions when necessary.

Developed almost three decades ago (Manja et al., 1982), the H<sub>2</sub>S test is based on measuring bacteria that produce hydrogen sulfide (e.g. *Citrobacter*, *Salmonella*, *Clostridium perfringens*) (Figure 1). In anaerobic aquatic environments, sulfate reduction by bacteria is the most important source of H<sub>2</sub>S. More specifically, the H<sub>2</sub>S strip measures the reaction of H<sub>2</sub>S with lead to form a black precipitate, iron sulfide. Given the low solubility of lead sulfide, this test can detect even small amounts of sulfide formation (Nikaeen et al., 2010). While the H<sub>2</sub>S method does not specifically test for standard indicator species such as total coliforms, fecal coliforms, or *E. coli*, a review of the literature indicates that the test detects fecal contamination (of both human and animal origin) with agreement between 68 and 96% (Sobsey and Pfaender, 2002).

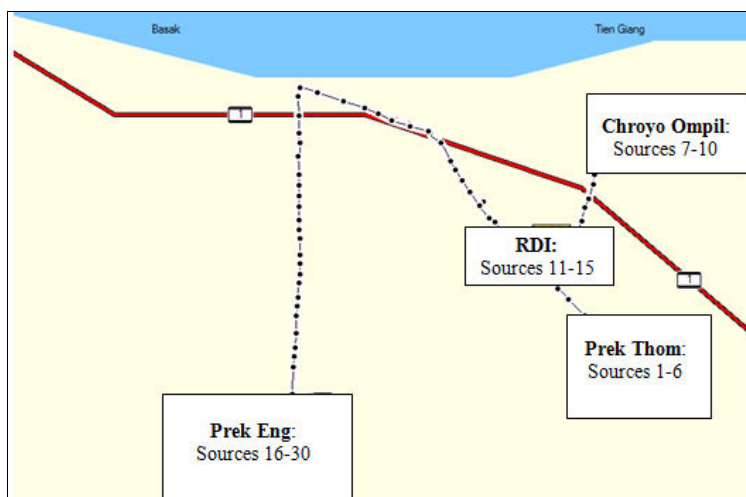


**Figure 1.** Summary of Coliform bacteria and H<sub>2</sub>S producing microbes.

The purpose of this study is to examine the effectiveness of H<sub>2</sub>S test strips for detection of bacterial contamination in water sources in Cambodia. Results were compared between the qualitative H<sub>2</sub>S strips and the quantitative membrane filtration tests. Ultimately, the effectiveness of the strips was statistically evaluated based on its sensitivity, specificity, predictive values for positive and negative, and accuracy.

## II. Materials and Methods

Field verifications of the H<sub>2</sub>S strips were conducted in 2009 and 2011. The 2009 samples were collected by RDI during six field investigations in Prey Salang and Weal Sbov, and the 2011 samples were collected by MIT D-lab in Prek Thom, Chroyo Ompil, and Prek Eng (Figure 2). More specifically, water samples were collected from a range of treated and untreated sources, including tube wells, dug wells, rainwater tanks, surface water bodies, and piped water systems.



**Figure 2.** MIT D-lab 2011 Sampling locations in Kien Svay, Cambodia

Between September and December 2009, a total of 159 water samples were collected and analyzed. The H<sub>2</sub>S strips used in the latter tests were either pretreated by autoclave or oven. Contrary to our expectation, the autoclaved and oven strips did not perform any better than the non-treated strips. As a result, for the 2011 samples, none of the strips were pretreated prior to experimentation. Of the 30 water sources that we tested, the first ten were duplicated. For this subset, we also ran 10 Pathoscreen tests (in 20 ml vials) to verify the qualitative H<sub>2</sub>S results. At the same time, we conducted two sets of the experiment—one in the field and one in the lab—to investigate how the H<sub>2</sub>S test will vary depending on temperature. To help us identify possible sources of contamination related to field sampling, control samples of boiled DI water were poured in the lab and in the field. For quality control purposes, a new control sample was collected in the field after every five sources. All samples were transported back to the lab in a dark and cool water container.

To quantify the accuracy of the H<sub>2</sub>S strips, results were compared against membrane filtration. Both tests were performed within 24 hours of the sampling date. The H<sub>2</sub>S samples were observed over a period of three days. The date and time of each observation was recorded as follows: (-) = no change; (+) = slight change, the water was partially gray; (++) = the water was partially black; and (+++) = the sample itself was noticeably black. In terms of the quantitative results, the membrane filtration test measured total coliform and *E. coli* in CFUs/100 ml.

### III. Results and Analysis

For both the 2009 and 2011 results, we compared the observed H<sub>2</sub>S presence with the total coliform results. Table 1 shows a summary of the collection dates and comparison data, such as the number of true positive, true negative, false positive, and false negative water samples. Ten samples were omitted from the 2009 analysis due to experimental error. Likewise, three samples were omitted from the 2011 analysis due to disagreements between the duplicates or field/lab results. As a result, only 149 and 27 samples were used in the correlation analysis, respectively (Table 2, Figure 3). More specifically, for the statistical analysis, we defined:

1. **Sensitivity** as the probability that the H<sub>2</sub>S strip will yield a positive result when the water sample is in fact contaminated: true positive/ (true positive + false negative);

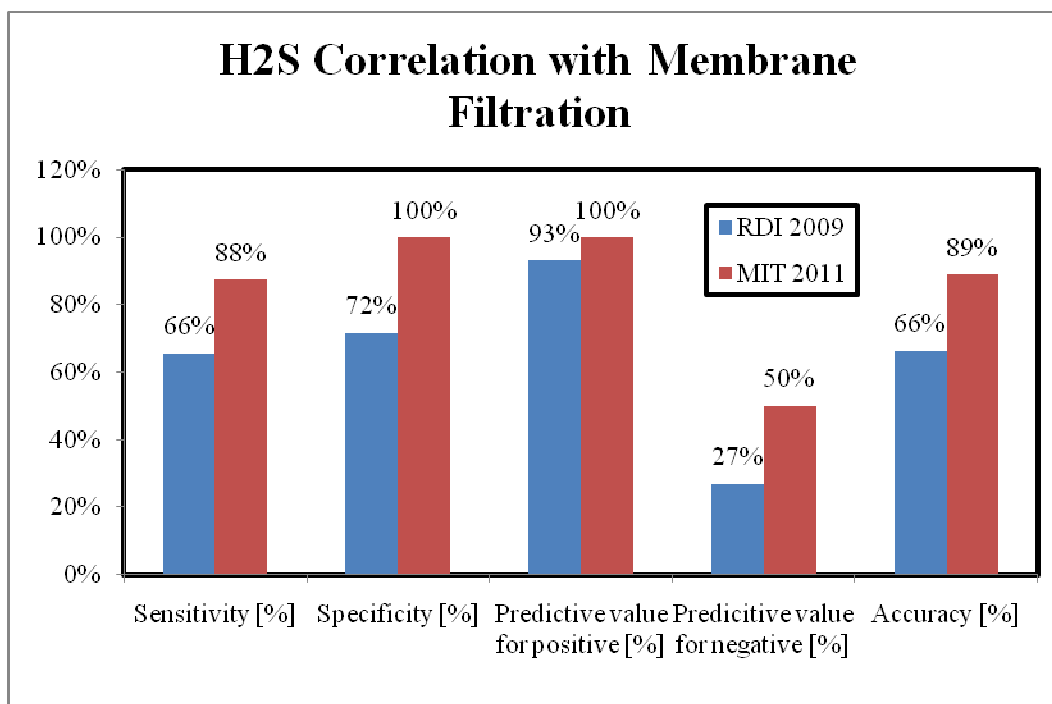
2. **Specificity** as the probability that the H2S strip will yield a negative result when the water sample is in fact not contaminated: true negative/ (true negative + false positive);
3. **Predictive value for positive** as the proportion of positive results that was correctly identified by the H2S strips: true positive/ (true positive + false positive);
4. **Predictive value for negative** as the proportion of negative results that was correctly identified by the H2S strips: true negative/ (true negative + false negative); and
5. **Accuracy** as the probability that the H2S strip will yield an accurate result: (true positive + true negative)/ (true positive + true negative + false positive + false negative).

**Table 1.** H2S Data Summary

|          | Date      | Strip Treatment | Test Assessment   |                    |                    |                   |                        | Total |
|----------|-----------|-----------------|-------------------|--------------------|--------------------|-------------------|------------------------|-------|
|          |           |                 | A (True Positive) | B (False Positive) | C (False Negative) | D (True Negative) | Further considerations |       |
| RDI 2009 | 29-Sep-09 | n/a             | 9                 | 1                  | 6                  | 4                 | 0                      | 159   |
|          | 6-Oct-09  | n/a             | 13                | 0                  | 4                  | 2                 | 1                      |       |
|          | 21-Oct-09 | n/a             | 10                | 1                  | 7                  | 1                 | 2                      |       |
|          | 29-Oct-09 | n/a             | 11                | 0                  | 4                  | 3                 | 2                      |       |
|          | 2-Dec-09  | Autoclaved      | 9                 | 1                  | 3                  | 3                 | 3                      |       |
|          | 2-Dec-09  | Oven            | 8                 | 2                  | 6                  | 1                 | 2                      |       |
|          | 8-Dec-09  | Autoclaved      | 11                | 1                  | 7                  | 1                 | 0                      |       |
|          | 8-Dec-09  | Oven            | 12                | 0                  | 6                  | 1                 | 1                      |       |
| MIT 2011 | 11-Jan-11 | n/a             | 21                | 0                  | 3                  | 3                 | 3                      | 30    |

**Table 2.** H2S Correlation with Membrane Filtration

|          | Date      | Strip Treatment | Sensitivity [%] | Specificity [%] | Predictive value for positive [%] | Predictive value for negative [%] | Accuracy [%]      |
|----------|-----------|-----------------|-----------------|-----------------|-----------------------------------|-----------------------------------|-------------------|
|          |           |                 | $A/(A+C)$       | $D/(B+D)$       | $A/(A+B)$                         | $D/(C+D)$                         | $(A+D)/(A+B+C+D)$ |
| RDI 2009 | 29-Sep-09 | n/a             | 60%             | 80%             | 90%                               | 40%                               | 65%               |
|          | 6-Oct-09  | n/a             | 76%             | 100%            | 100%                              | 33%                               | 79%               |
|          | 21-Oct-09 | n/a             | 59%             | 50%             | 91%                               | 13%                               | 58%               |
|          | 29-Oct-09 | n/a             | 73%             | 100%            | 100%                              | 43%                               | 78%               |
|          | 2-Dec-09  | Autoclaved      | 75%             | 75%             | 90%                               | 50%                               | 75%               |
|          | 2-Dec-09  | Oven            | 57%             | 33%             | 80%                               | 14%                               | 53%               |
|          | 8-Dec-09  | Autoclaved      | 61%             | 50%             | 92%                               | 13%                               | 60%               |
|          | 8-Dec-09  | Oven            | 67%             | 100%            | 100%                              | 14%                               | 68%               |
|          | Mean [%]  |                 | <b>66%</b>      | <b>74%</b>      | <b>93%</b>                        | <b>27%</b>                        | <b>67%</b>        |
| MIT 2011 | 11-Jan-11 | n/a             | <b>88%</b>      | <b>100%</b>     | <b>100%</b>                       | <b>50%</b>                        | <b>89%</b>        |



**Figure 3.** Mean results for H2S and membrane filtration tests collected in 2009 and 2011.

#### IV. Discussion

Based on our analysis of the 2009 and 2011 results, the overall accuracy of the H2S strip improved from 66 to 89%. When we examine the individual statistical parameters, we also observed improvements in the sensitivity (66 and 88%) and specificity (72 and 88%) of the H2S test. Our result for the predictive value for positive (93 and 100%) confirms that the H2S method is very reliable for detecting bacteriological contamination. In cases where the membrane filtration test did not detect any total coliform or *E. coli* bacteria, false positive results may be indicative of the presence of non-coliform sulfate-reducing bacteria (e.g., *Salmonella*, *Clostridium perfringens*, etc...). The number of false positives is of less public health concern when compared against the speed, simplicity, and low cost of the test.

However, our predictive value for negative (27 and 50%) suggests that caution must be used when evaluating the negative H2S results. The high number of false negatives is disconcerting. Based on the 2011 data, the false negatives are mainly a result of the strip's insensitivity to low bacteria count (i.e., < 100 CFUs). While the Pathoscreen test supported the H2S results, it was equally insensitive to low bacteria count. To remedy this, we recommend further studies to increase the sensitivity of the strips, such as adding citrate to the H2S media. To improve the predictive value for negative, we also recommend testing more samples to buffer tests that may have been skewed due to a limited number of true negatives and false positives.

In terms of the H2S reaction rate in the field and in the laboratory, the 2011 results show that the diurnal temperature variation did not affect the overall reading after three days. Except for the low coliform samples, results for the duplicates were consistent as well.

False negatives are the most concerning limitation to the H2S test. For example, recommendations based on a false negative may result in continued consumption of contaminated water. Other possible

limitations or sources of misinterpretation exist. Since a black precipitate is the end point of the H<sub>2</sub>S test, there may be concern for other dark precipitates forming. However these precipitates should either be evident immediately at the onset of the test or not at all, as they are unlikely to form as a result of microbial activity. For this reason, it is important to perform a visual check for a quick reaction after a few minutes or an hour of incubation. Additionally, if sulfates are already present in the water, this could result in a false positive reading. For this reason, an early visual check of the samples is also recommended. In conclusion, while the H<sub>2</sub>S test may not be mature enough to replace conventional methods, we would still recommend it as a valuable tool in screening water supplies for bacteriological contamination.

## Bibliography

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## Appendix: Pictures



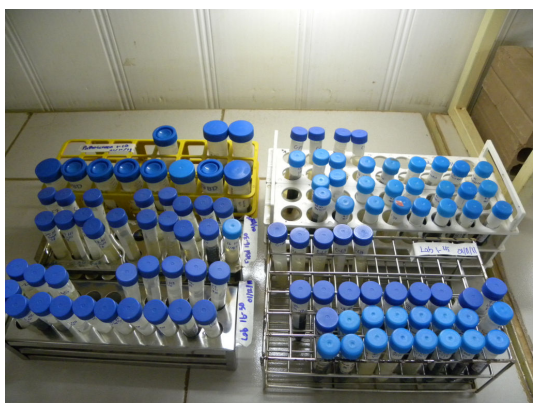
**Image 1.** Empty 15 ml vials with H2S strip



**Image 2.** Sampling in Prek Thom



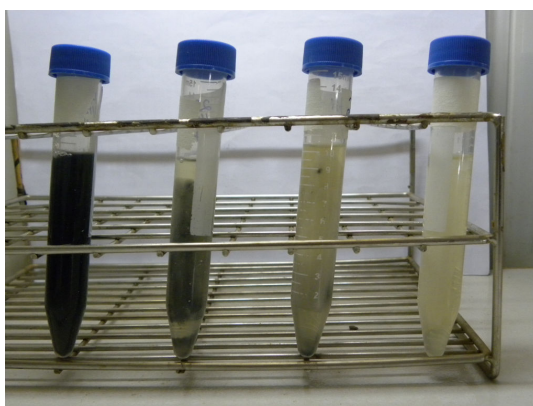
**Image 3.** Water collection in Prek Eng



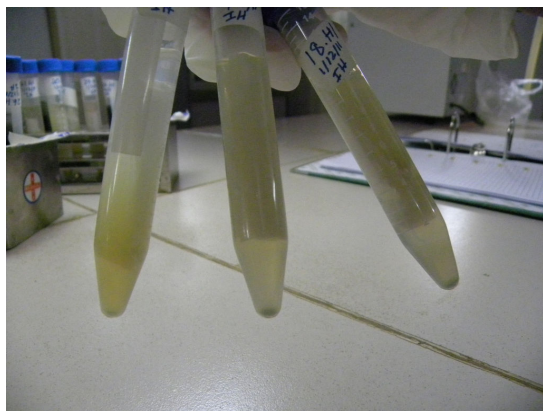
**Image 4.** H2S vials for field and laboratory incubation, including duplicates and Pathoscreen vials (20 ml)



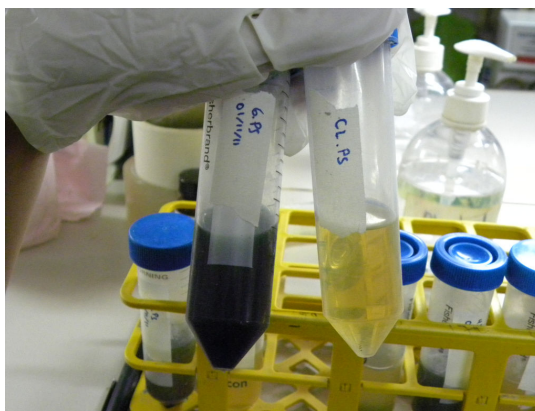
**Image 5.** Field incubation of H2S vials



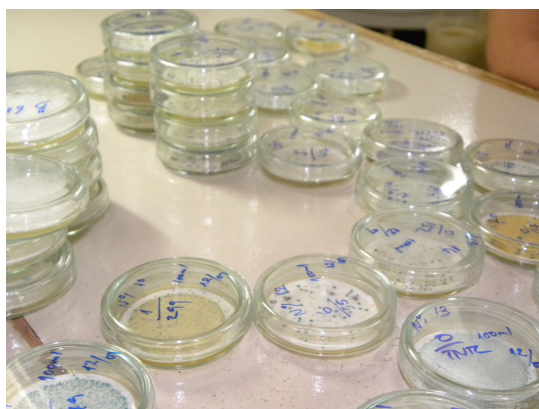
**Image 6.** Reading the samples from left to right: (+++), (++) , (+), (-)



**Image 7.** Samples with low coliform count



**Image 8.** Pathoscreen result: contaminated vs. control



**Image 9.** Membrane filtration plates