MIT D-Lab
January 20, 2011

Procedures for the H2S test

A. Making H2S strips

1. Clean all test tubes by soaking in a 5% bleach solution and washing with detergent. Let the vials air-dry before using the autoclave.
2. Pour 100 mL of boiled DI water into a clean 250 mL Erlenmeyer flask using a graduated cylinder. Place the flask on a magnetic stirrer and turn it on.
3. Measure out each chemical according to the formula below using a balance (AE model, 0.005g precision) and aluminum weight boats. Be sure to clean any scooping device in between measurements with alcohol and DI water.

H2S Media Formula

Ingredient	Amount
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

Figure 1. H2S media solution

4. Carefully pour each chemical into the flask. To minimize chemical loss, it may be best to weight the peptone last. It might be most convenient to use a 5ml syringe to measure out the detergent—avoid air bubbles by carefully inverting the syringe. Keep the solution on the stirrer until all the chemicals are completely dissolved. This media can be stored in a fridge for a couple of days.
5. Taking any type of absorbent material, place a measured quantity of media on the paper. For example, a 10 ml water sample would need a paper strip that contains 0.5 ml of media. In the picture below, 6 ml of media was dripped evenly onto a two-ply paper towel and dried in an oven at ~ 55°C for 30 minutes. Afterward, the paper towel was cut into 12 uniform strips (i.e., 0.5 mL of media per strip).
6. These reagent-impregnated strips can be stored dry for several months (in an envelope or preferably a zip-locked bag) – until ready for use.
7. Prior to experimentation, fold and insert one strip into a clean sample bottle and capped it tightly. The tubes or bottles should be stored in a dark place until ready for use.

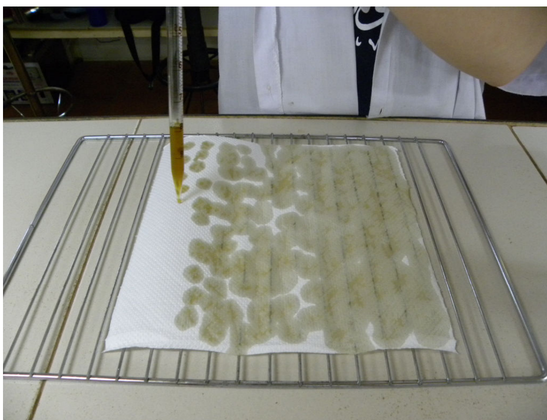

Figure 2. H2S strip making

Figure 3. Strip drying in oven

Figure 4. Cutting the strips (0.5 ml each)

Figure 5. Finished strips in 15 ml tube

B. Field Sampling

1. Label each container with the sample number, date and initial of analyst. Record corresponding information on data collection sheets and include additional comments on water conditions if available (odor, color, or any observations that may have influenced the water quality).
2. Prior to sampling, pour a 10 mL control sample of DI water in the lab and in the field. For quality control purposes, pour a new control sample after every five sources or so. It is also recommended to pour controls into sample collection bottles. This will help identify possible contamination related to the transport of the samples.
3. Water collection from the different sources:

   i. From storage containers, wells, or natural waters:

      a. Rinse the utensil that is normally used by the consumer or water collector to collect the sample.

b. Using the utensil, collect and transfer the appropriate amount of water to the collection bag. Be sure that there is enough water for the membrane filtration (100mL) and H2S tests (10mL), as well as any duplicates.
c. Then carefully place and transport all the water samples in a dark and cool container (4°C for membrane filtration samples).
d. Rinse all utensils with DI water before proceeding to the next source.

ii. From tap water:
a. Let the water run freely for about 15-20 seconds or pump water for a few minutes.
b. Place the opened water collection bag under the tap and collect the appropriate amount.

4. Carefully place and transport all the water samples in a dark and cool container (4°C for membrane filtration samples).
5. Rinse all utensils with DI water before proceeding to the next source.

(Note: There will be slight change in the color of the sample to a pale yellow due to the color of the reagent, which is normal).

C. 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 fecal origin. The speed of the reaction may be indicative of the density of organisms present, i.e. the quicker the reaction the higher the number of fecal organisms. For example, a slight color change (+) on day three indicates a lesser risk than a strong (+++) change on day 1. However, other factors such as incubation temperature or strip concentration could also affect the rate of the H2S reaction.

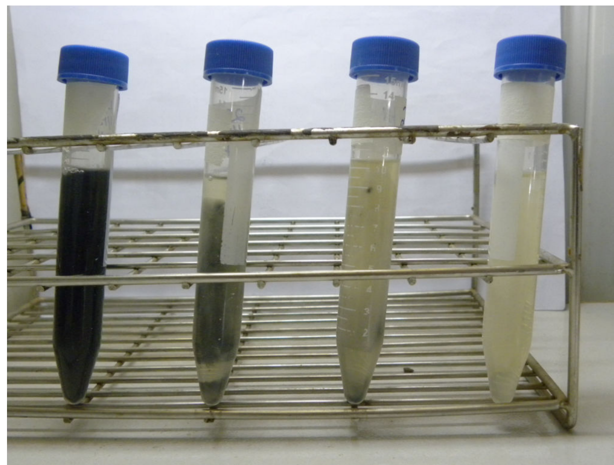

Figure 6. Reading samples

D. Disposal and Cleaning

1. After reading results, tests should be collected for proper disposal and cleaning.
2. Open vials carefully and add bleach to disinfect the vial contents. There may be a strong smell from the hydrogen sulfide gas.
3. The liquid can be poured down the drain with running water.
4. The paper should be bleached and sealed in a bag until it can be incinerated.
5. The reusable vials should be soaked in 5% bleach solution overnight, air-dried, and then autoclaved before being used again.