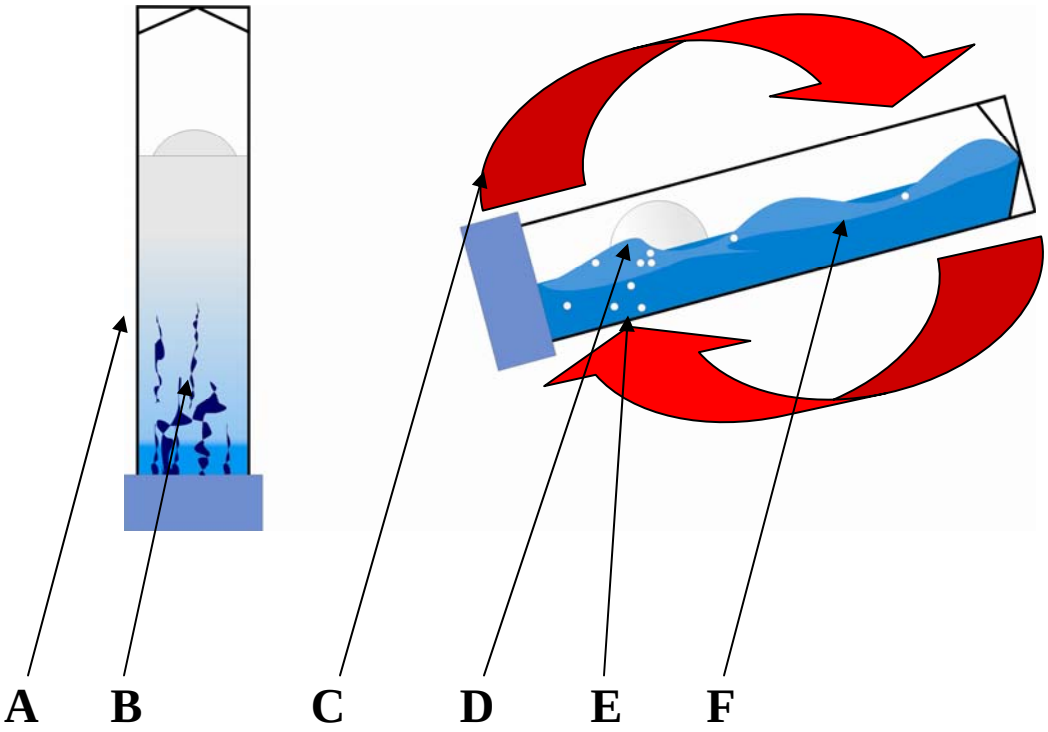
DBHSOPO5

Standard Operating Procedure for setting up HAB-BART tester

Regardless of whether a water, oil or soil sample is employed there is a standard operating procedure for charging the tester with the methylene blue. Because methylene blue does not have an adequate storage capability in the liquid form or as a crystallized material associated with other chemicals, it is dried in the inner cap of the tester. Here the methylene blue maintains specifications for at least four years when stored in the standard foil pouches. To set up the HAB-BART tester this methylene blue has to be dissolved into the liquid (i.e. water sample or sterile phosphate buffer) and then shaken to aerate the liquids to allow saturation with oxygen from the headspace gases. It is very important to ensure oxygen saturation since this ensures a highly oxidative condition for the start of the test. The standard procedure is summarized in Figure Eight. There are two stages in this activity. First the charged tester is placed upside down for thirty seconds so that the dried methylene blue in the cap dissolves and diffuses into the water. Second, the tube is shaken by a rotating wrist action five times which causes the ball to travel the full length of the tube ten times causing the air from the headspace atmosphere to be dispersed into the liquid to reach saturation. This agitation also disperses the microorganisms throughout the liquid.

Figure Eight, Preparing a HAB-BART tester for a test



This figure shows the manner in which the methylene is dispersed into a HAB-BART tester. First the tester is placed upside down for 30 seconds (A). Here the methylene blue dried in the cap diffuses into the liquid commonly as a series of dark blue columns (2). After 30 seconds of standing upside down the tester is now rotated (C) by a vigorous wrist action five times causing the ball (D) to move from one end to the other end of the tester ten times. This causes the air to get mixed into the liquid (E) so that the liquid becomes saturated with oxygen and the liquid develops an even blue color. The tester is now ready.

Recommended Protocol for Water, Condensate and Waste Water Samples

Cautionary conditions relating to water sample collection and storage

There are standard procedures for the collection of water and waste water samples but these are not so well defined for other waters. Essentially Heterotrophic bacteria tend to be able to survive 2 or 3 days in water samples with no significant loss in activity if the samples are kept over ice or in a refrigerator at $<8^{o}C$. There are a number of basic considerations to ensure precision and validity in the final interpreted data. These can be summarized as needing to include the following conditions:

- If the sample is going to be tested within four hours of collection then the sample may be allowed to acclimatize to room temperature at a site away from direct sunlight in a place where there are no vibrations or disturbances of any type.
- If a water sample is taken from a continuous stream flow then the sample should be taken at midpoint as far as possible to avoid contamination from any growth occurring on the walls of the conduit.
- If it is desired to examine the types of heterotrophic bacteria growing attached within the conduit with which the water is in contact, then changing the environmental conditions through stopping or radically changing the flow rate will cause stress amongst the attached bacteria including the HAB. There is likely to be more releases of HAB into the water.
- If a water sample has to be collected longer than four hours prior to testing then the sample should be cooled down over ice to approximately $4^{o}C$. Water samples for HAB determinations can be kept for 72 hours in this state with no significant changes in activity.
- Water samples should always be kept in sterile containers whenever possible to reduce the risk of contamination.
- In the event that sterile containers are not available in the field for on-site testing then the outer vial of the HAB-BART field tester may be used to collect the sample.
- It is not necessary for the sample container to have a chemical tablet to neutralize residual chlorine since: (a) the heterotrophic bacteria do have a resistance to the normal levels of residual chlorine in water; and (b) the HAB-BART incorporates a chlorine neutralizing chemical to eliminate any potential inhibition.
- It is not necessary to completely fill the sterile sample container with the sample water since this would cause some restriction in the available oxygen since it would be used up through indigenous respiration leading the a more reductive

ORP which would reduce possible overgrowth by aerobic bacteria and reduce the stress on the oxygen sensitive heterotrophic anaerobes .

- Cleanliness should be imposed in all stages of the collection and handling of the sample and rigorous efforts need to be applied to assure no casual contamination of the sample in any manner.
- It is recommended that latex gloves be worn by the operator when working with the HAB-BART testers.

There are two different types of HAB-BART tester are the: (1) field tester; and (2) laboratory tester. The field tester is designed for use away from the laboratory and can be charged with the sample close to the sampling site and monitored in the field at a suitable temperature. This type of tester includes an outer vial which surrounds and protects the inner vial in which the test is actually performed. The other type of HAB-BART tester is meant to be used in the laboratory under normal conditions for the handling of microbiological materials. This tester includes the essential inner tester necessary for undertaking the HAB-BART test but without the use of the outer vial. The field tester therefore provides additional protection for the user from potential leakages of odorous gases or cultured fluids that may occur from the inner tester.