Protocol DBHFWO5

Using the HAB-BART (Field version) tester to test for HAB in water

Filling the HAB-BART tester involves a sequenced approach that ensures that there is no casual contamination of the tester and that a suitable volume of sample has been added. Generally when the field HAB-BART is going to be used, it would be used in a field setting (rather than laboratory) and will be read visually on a daily basis as far as is conveniently possible. Where greater precision is desirable then it is recommended that the laboratory tester be employed along with the HAB-BART reader systems Instructions for the charging the HAB-BART field tester is described below:

The field tester comes in packs of three each separately sealed within an aluminum foil pouch. To remove a field tester from the pouch, locate the "tear down" tab on one end of the pouch, grip it tightly and pull it down over the pouch. This will exposed the field tester that can then be removed. Make sure that there is a clean flat surface to place the tester in a place where it is not likely to be knocked over.

The container containing the sample to be tested should be placed next to the field tester on a clean surface and it is important to make sure that there is not likely to be any strong winds or air currents that could bring dust into the area where the test is going to be set up. It is recommended that latex gloves be worn during the setting up of the HAB-BART testers to reduce the risk of contamination. Follow the sequence of activities as described below:

(i) Label the top of the cap on the outer vial of the field tester with the essential information to recognize the sample (e.g. name, date, time) using a black fine point permanent marker. Do not write on the plastic walls since this might seriously impair the ability of the operator to recognize positive reactions. Make sure that the ink from the marker is dry before attempting to charge the field tester. If this is not done then sample information may become smudged and unreadable.

(ii) When ready to charge the field tester with the sample, the first step is to unscrew and remove the outer cap of the tester. It should be noted that the act lifting up the outer cap causes the inner tester vial to be lifted up as well.

(iii) As the outer cap is lifted up clear of the outer vial then gently remove the inner tester from the outer cap and place that cap down on a clean surface without turning it over and place the inner tester next to the sample to be tested. It is strongly recommended that charging of the tester be done one at a time to reduce the potential for inadvertent mixing of the samples and testers.

(iv) Unscrew the inner cap from the inner tester and place the cap down on a clean surface without turning it over. Note this cap is not labelled with the sample information and so it is important to remember to do one test at a time to prevent mixing up of samples. Note also that the contents of the inner HAB-BART tester are now exposed to possible contamination from the outside environment and so the next steps should be done quickly. Unscrew the cap from the sample container and slowly pour sufficient water into the inner tester vial to bring the water level up to the fill line indicating 15ml of sample has been added. The ball will float up on the

rising water column and the water level is observed to be at the fill line and against the ball. When pouring water into the inner tester vial, every effort should be made to direct a stream of the sample water over the center of the ball rather than allow it to trickle down the side. Care should be taken to ensure that the final water sample level is to within 2mm of the etched fill line on the inner vial of the BART tester. For the effective use of the field tester, maximum tolerance for error for filling the HAB-BART tester is 5% so that the amount of water sample being tested falls within the range of 14.25 and 15.75ml. It should be noted that the sample container retains a headspace of air over the sample and so some oxygen will diffuse down into the liquid sample. To assure that there is oxygen in the sample along with a dispersion of any settled or particulate materials within the sample it is recommended that the sample container be inverted five times before being used to charge the field tester. This assures a better level of precision since the oxygen concentration at the start of the test would be at a saturated level and the possible bacteria of interest are more likely to be evenly dispersed throughout the sample.

(v) Once the inner tester vial has been charged, immediately screw back the inner cap down on the vial. This cap does not need to be screwed down hard. Do not shake or disturb the contents of the inner tester vial in any manner. Lift the outer cap without turning it over and clip the inner tester vial back into the underside of the outer cap. Now grip the outer cap (inner charged tester attached) should now be tightly held inside this cap). Lower the inner tester back down into the outer tester vial. Screw the outer cap down firmly but without excessive force. The charged field tester may now be moved to a safe place for periodic observation. In moving the charged tester remember that the test has started and that complex diffusion fronts for oxygen (moving downwards) and nutrients (moving upwards) are occurring and that severe movement may affect the precision of the tester.

(vi) Testing begins as soon as the sample has been added. If the sample has a much lower temperature than the temperature at which the tester will be maintained (e.g. room temperature) then there is a probability that excess oxygen might be released into the culturing tester causing gas bubbles to form on the walls. <u>This is not a positive reaction</u>. To minimise this risk, the sample should be given time acclimatise to the temperature at which the testers will be monitored (e.g. room temperature). This acclimatization would be dependent upon the sample bulk volume but should, under normal conditions, take no longer than one hour.

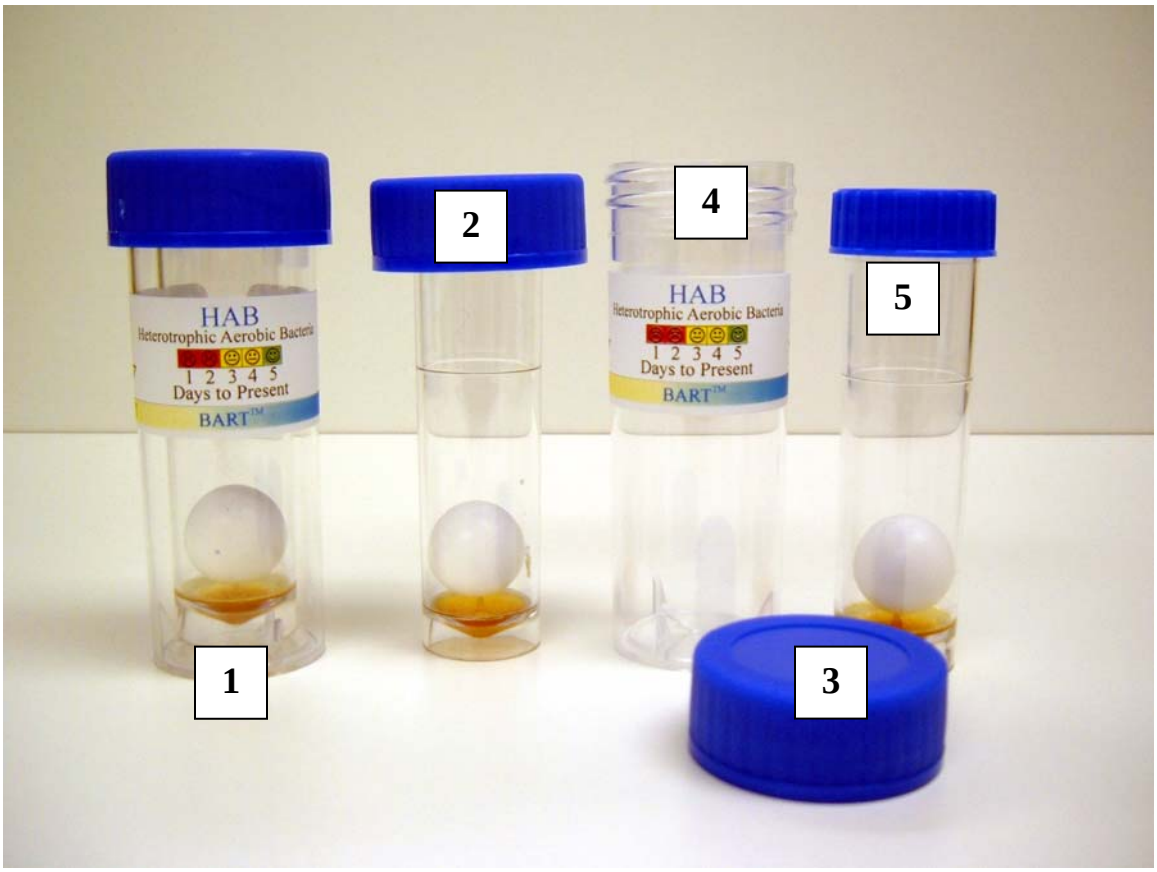

Plate One, HAB-BART Field Tester. The sequence for preparing the HAB-BART field tester for testing is summarised as: (1) take a field HAB-BART tester; (2) unscrew the cap and place on clean dry surface; (3) remove the inner tester vial from the cap; (4) put the outer vial aside until required at the end of charging the tester with the sample; and (5) fill the inner tester vial with the 15mL sample (to the fill line). Note these activities are undertaken in rapid succession leaving the operator with items 2, 4 and 5. The next plate shows the method for charging the field tester with sample.

(vii) Incubation temperature commonly recommended for the field HAB-BART tester is room temperature which is considered ideally to be between 21 and 25^{o}C away from direct sunlight that could cause internal heating of the tester. If the temperature goes above 25^{o}C then there is most likely to be an increase in the rate of iron related bacterial activity and the time lags for given population sizes can be expected to drop. This would cause higher populations of HAB to be predicted. If the temperature drops below 21^{o}C then this may commonly slow down the level of bacterial activity causing losses in precision (where duplicate tests are performed) and much lower predicted populations.

(viii) Observation for the occurrence of the UP or DO reactions in the tester should ideally be performed daily until day 6 and then again on days 8, and 10 if no

reactions are observed. Critical days for the observation of activities and reactions are days 1, 2 and 3 along with such other days up to day 10 if the objective is to obtain a qualitative and/or quantitative assessment from the sample in the tester. Remember that the only significant reactions are the UP and DO reactions and that only one of these reactions can occur, There are no subsequent significant reactions for the HAB-BART tester.

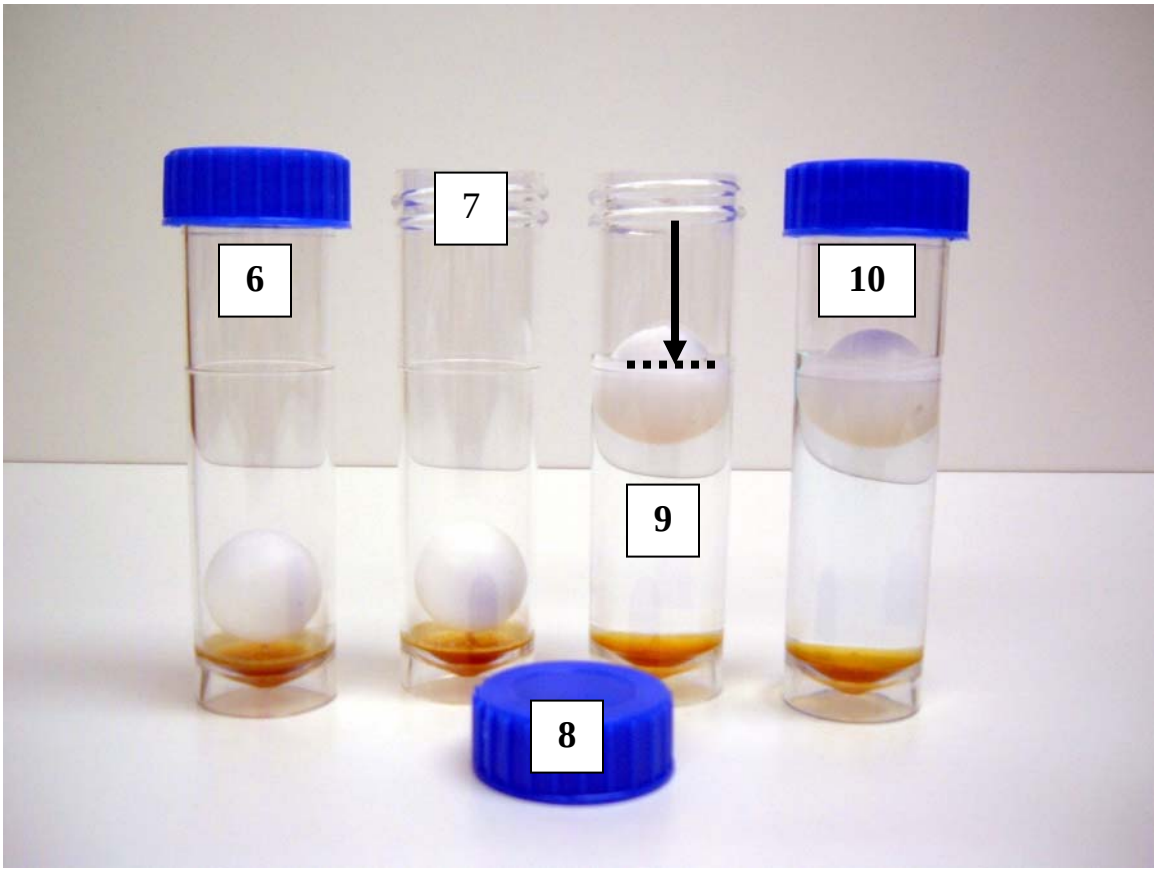

Plate Two, Filling the Inner Vial of the HAB-BART Field Tester In the above plate the sequence for charging an inner HAB-BART tester (6) as requiring the following steps: (7) unscrew the cap; (8) place the cap down on a clean surface without turning the cap over; (9) carefully fill the tester vial with the sample until the water level is at the same level as the fill line, note that the ball floats up as the water level rises; and (10) screw the inner cap back onto the vial without using force. Once this has been done then the outer cap can be positioned over, and pressed down onto, the inner test vial that will now become seated into the outer cap. Once this has been done then the outer cap (with the inner tester attached can be returned to the outer vial and screwed down. The test for the presence of iron related bacteria has now started.

(ix) To observe a tester for significant reactions then it is important to follow the same procedure each day (and at the same time of day as the test was started). This

procedure would consist of lifting the tester up towards a light such as a fluorescent light fixture or the northern sky during daylight hours. The ideal position to view the tester is to place it directly in front of the light source at arms length. The culturing tester would begin by having a blue color which would, when there is a positive, move to a clear, clouded or pale yellow color. See Figures four and five for the reactions patterns typical of a UP or DO reaction.

(x) Finished tests should be disposed of using one of the techniques recommended in below.