



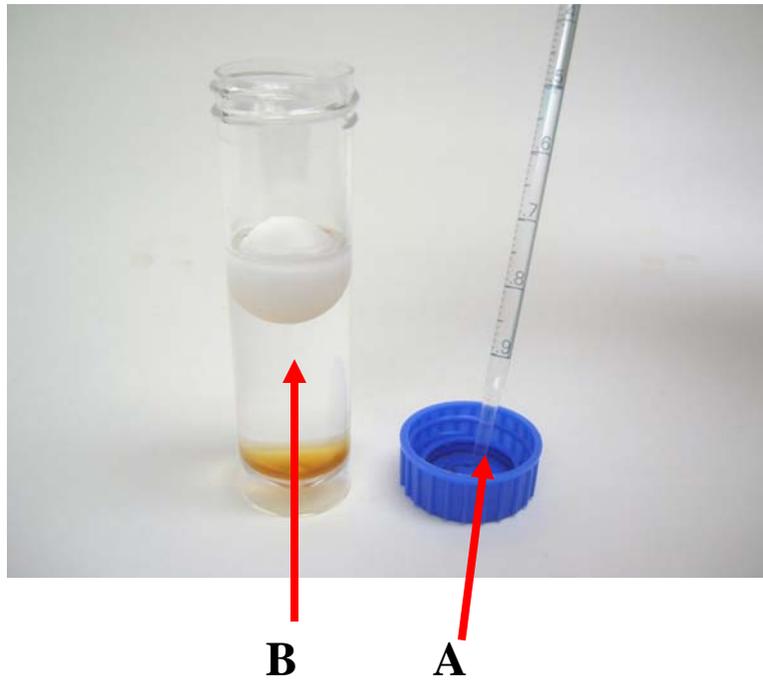
## **Protocol DBHSSOPO5**

### **Method and Standard Operating for Conducting HAB-BART tests In Brackish and Saline waters**

This protocol is a supplementary protocol to DBHSSOPO5 which specifically addresses the method for mixing the ORP indicator, methylene blue, into the water sample to be tested using the HAB-BART system protocols described in protocol document (HAB-BART protocol 0305). This methodology discusses the modifications to the standard operating procedures (DBHSSOPO5) if the water sample contains more than 3% salt up to saturation. Here, the standard method of inverting the HAB-BART tester once charged in order to assure that the methylene blue dissolves evenly in the sample will not function efficiently and the resultant color in the solution may be a shade of green or, at higher salt concentrations, the methylene blue will not dissolve in the water sample. This protocol calls for the methylene blue to be dissolved in the inverted cap using 0.5mL of sterile distilled water prior to the inversion of the tester. To undertake this part of the DBHSSOPO5 protocol then the following modifications are necessary to assure precision in the testing procedure:

1. Remove the cap from the tester and place on a clean dry surface with the inside facing upwards. This exposes the dried methylene blue crystals that are present within the cap.
2. Using a sterile pipette, dispense 0.5mL of sterile distilled water into the center of the upturned cap so the fluids fill the central base of the cap. Note that the methylene blue will now dissolve in the water to form a dark blue solution. Leave for 30 seconds.
3. Tip the fluids in the cap carefully into the HAB-BART making sure that all of the contents come to sit around the floating ball in the tester. There will now be a blue band of methylene blue solution suspended in the top of the sample to be tested.
4. Screw the cap down firmly onto the HAB-BART tester. The tester is now ready for the five rotations that will mix the methylene blue evenly into the sample being tested and assure that the sample is fully oxygenated.
5. Continue using DBHSSOPO5 to conclude the set up of the test and proceed with such other protocols as may be relevant to the sample testing procedure.

**Plate One, 0.5 mL of sterile water is added (A) to the inside of the inverted cap in order to pre-dissolve the methylene blue crystallized in the cap before it comes into contact with high concentrations of salt that would be in the sample (C).**



**Plate Two, Once the 0.5mL of water has added then the cap is left for 30 seconds to dissolve the methylene blue and the contents of the cap are tipped into the tester.**



Once the methylene blue has been added to the tester, a blue band will appear (4 to 8mm) around the ball and down into the sample. Screw the cap firmly back onto the tester the tester and rotate the tester five times to ensure oxygenation of the sample and that the methylene blue has mixed into the sample (see also protocol DBHSOPO5 for more details of this procedure).

### **Claims**

This protocol is suitable for the examination for heterotrophic bacteria in waters that have a salt content of between 3% and saturated. It is recommended that before the HAB-BART tester is placed into a reader then the outside of the tester be wiped down with a clean dry paper cloth to remove any droplets of potentially salt rich water that may have gathered there. Such salt solutions, when dried, could create significant corrosion problems for the monitoring equipment and the surrounding environment.

### **Further information**

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HAB-BART system, 2003