Protocol DBIFWO5

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

Filling the IRB-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. Instructions for the charging the IRB-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 IRB-BART testers to reduce the risk of contamination. Follow the sequence of activities as described below:

(i) Label the outer red cap 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 walls since this might seriously impair the ability of the operator to read 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 lifting up the outer cap causes the inner tester vial to be lifted up as well. This is displayed in the figures shown below.

(iii) As the outer cap is lifted up clear of the outer vial then gently remove the inner test vial from the cap and place the cap down on a clean surface without turning it over and place the inner tester next to the sample. 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 vial 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 IRB-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 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 IRB-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 inner test vial should now be tightly held inside this cap) and lower it 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 fluids causing gas bubbles to form on the walls. <u>This is not a positive reaction</u> and should not be confused with the FO (foaming) reaction that will occur around the ball. 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.