Protocol DBHLS05

Using the HAB-BART (laboratory) tester to test for HAB in soil

The HAB-BART tester offers some unique characteristics that are the result of the requirement for methylene blue to be mixed into the water and the need to create a saturated oxygen (oxidative) regime. This means that an additional stage is involved in the testing of soils to allow the addition of methylene blue to the test protocol. Not only has that but soil testing required a different approach. This is because it is not possible to simply dispense the soil into the BART tester without the soil becoming perched around the ball. If this were to be done then it would also disturb the effective generation of the redox gradient essential to the functioning of the HAB-BART tester. It is therefore important to remove the ball from the tester before adding the soil sample. Addition of soil is shown in Plate three following the recommended manner in which the ball should be removed from the tester prior to the adding of the soil.

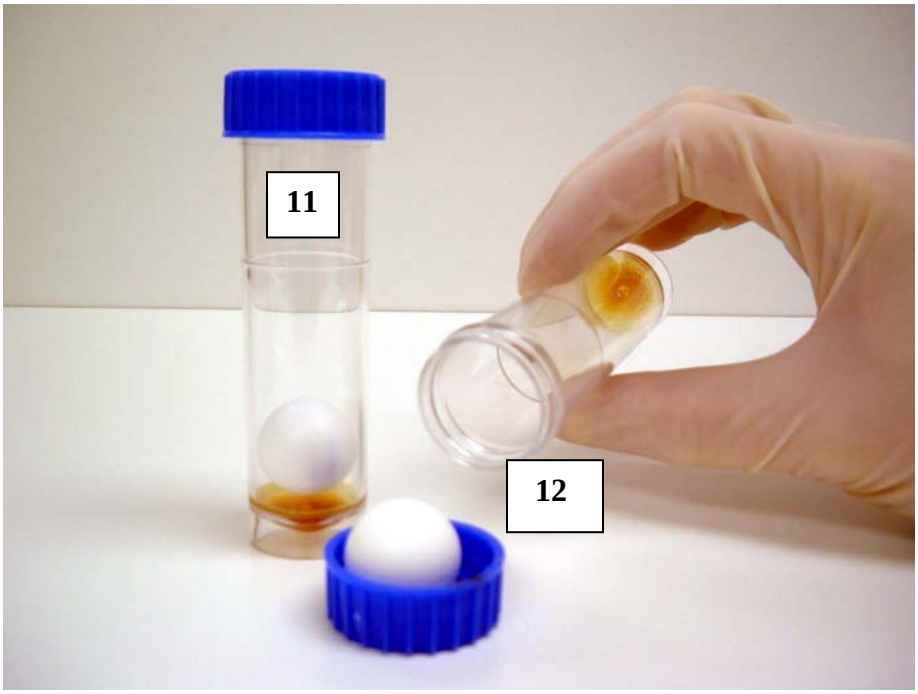
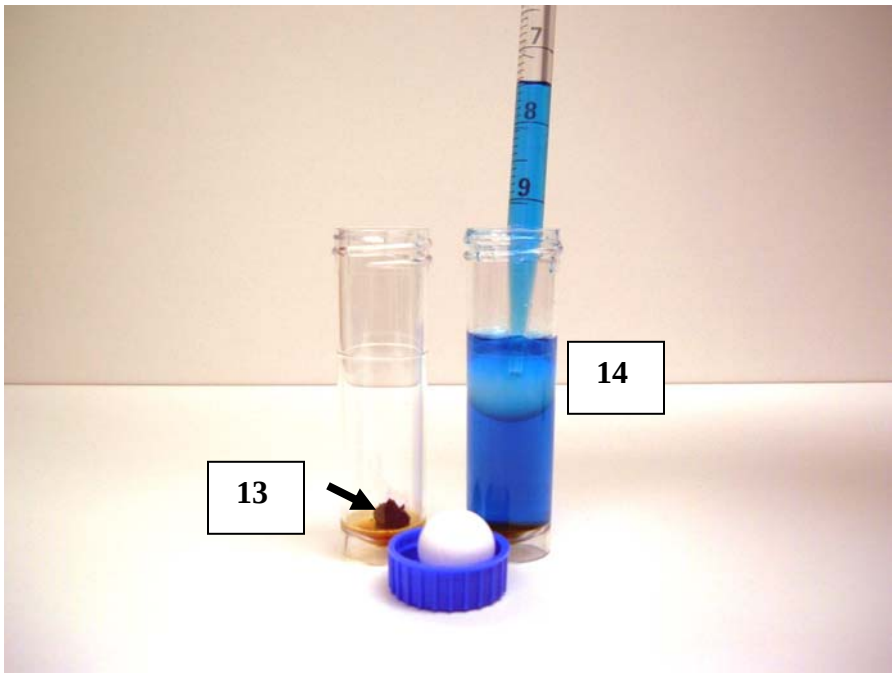


Plate Three, Removal of the ball from the tester to allow soil testing.

To prepare to add the soil sample for testing, the screw cap (11) has to be removed from the laboratory tester and place with the inside facing upwards. This inside surface is sterile and so should not be contaminated in any way. The ball is now rolled out of the

tube into the cap (12) and the tube placed upright again. It is now ready for the soil sample to be added and the test started.

In plate four, the method for adding the soil to the laboratory tester is outlined to allow the start of the testing.



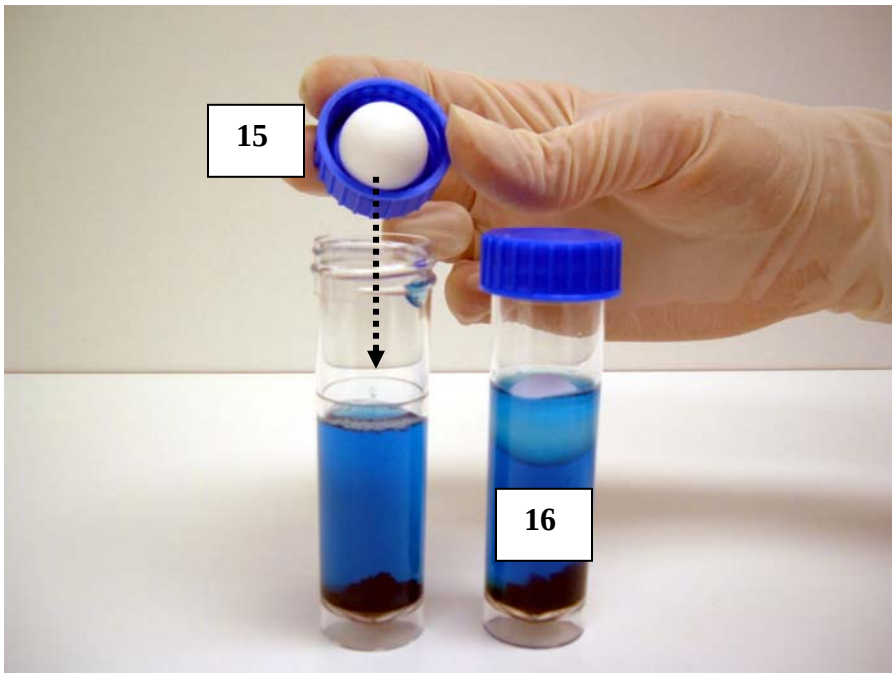


Plate Four, The addition of the soil sample to the HAB-BART laboratory tester.

0.1g of soil is added using a clean spatula to drop the soil onto the floor of the tester (13). It should be noted that a sandy soil may require 0.5g of soil to be used in order to get effective testing. If 0.1 g of soil is used then the population calculated using the HAB-BART system would need to be multiplied by 10, if 0.5 g of soil is used then the population should be multiplied by 2 to get an accurate population count. Once the soil has been added then 17.5 mL of sterile water is added to the second tube (16) in the soil test procedure. This tube is a second HAB-BART tester that allows the mixing of sterile water with the methylene blue to achieve an oxidative state prior to the admission of the liquid (14). To achieve an oxidative state procedure DBHSOP is used on the second HAB-BART tester. Once the sterile water has been oxidized (turned blue) then the 15 mL of the oxidized methylene blue is pipetted into the tester containing the soil sample. The ball can now be rolled back into the tube (15). Once the tube is capped again (16) then the test can start at the appropriate temperature. Incubation can be at room temperature (21 to 25^{o}C) but other temperatures can be selected since different incubators are likely to be available. Faster reactions and activities can be achieved sometimes by incubating the testers at 27 to 29^{o}C but this will cause the time lags for a given population to shorten. BART QuickPop software has interpretation methods to project the population when this higher temperature is used but a summary table below gives some of the time lag to p.a.c. / ml population relationships.

It should be noted that:

1. Two HAB-BART testers are required to perform one test on a soil sample; and
2. Soil with a high fraction of oil is likely to create odd effects in the tester during incubation. When there is significant hydrocarbons (i.e., >1% by weight) the soil particles may become mobilized and float up into fluids.