

## **Protocol DBILSO5**

### **Using the IRB-BART (laboratory) tester to test for IRB in soil**

Soil samples require a different approach since it is not possible to simply dispense the soil into the BART tester. If this were to be done then the soil particles would perch around the ball and disturb the effective generation of the redox gradient essential to the functioning of the tester. It is therefore important to remove the ball from the tester before adding the soil sample.

To prepare to add the soil sample for testing, the screw cap has to be removed from the laboratory tester and placed 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 and the tube placed upright again. It is now ready for the soil sample to be added and the test started.

0.1g of soil is added using a clean spatula to drop the soil onto the floor of the tester. 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 IRB-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 15 mL of sterile saline (0.1% NaCl) should be added followed by the ball being rolled back into the tube. Once the tube is now capped again then the test can start at the appropriate temperature. Incubation can be at room temperature (21 to 25°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°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. Remember that the IRB-BART only functions giving the full range of reactions at incubation temperatures up to 30°C.

It should be noted that 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. This may also be accompanied by heavy gassing. Such activity may disrupt the observation of the reactions and activities in the tester and so the testing of high oil content soils is not recommended.