Chapter 5

Sample Preparation

5.1 Preparation of Samples

BART testers can be used for water samples directly by the admission of 15ml of sample added directly to the tester (this is referred to as “charging the tester). However there are occasions when the sample needs dilution to allow the tester to function properly. These conditions are summarized in Table 5.1.1.

Table 5.1.1, BART analysis of turbid waters, soils and growths

Sample type	Amount	Diluant volume	M.	Dil.	Correction Factor
Turbid waters	1.5mL	13.5mL	A	SDW	x10
Black waters	0.15mL	14.85mL	A	SDW	x100
Sandy loam	1.5g	13.5mL	B	SDW	x10
Loam	0.5g	14.5mL	B	SDW	x20
Clay loam	0.1g	14.9mL	C	SDW	x150
Concretion	1.0g	14.0mL	B	SDW	x15

Note: Amount is given in ml or grams of original sample; Diluant volume is the volume of diluant used in mL; M. is the method applied (see below); Dil. is the diluant used; Correction factor is the multiplication factor to be applied to the population calculated from the time lapse conversion as pac which might be in pac/mL or pac/g depending on the sample source; turbid waters are those waters too thick or colored to allow clarity sufficient to recognize the testers reactions for example black waters are those waters that appear to be black but do not necessarily contain a high particulate content; loams are defined as being predominantly sand, or as a mixture of sand, silt and clay, or a clay loam; concretion defines microbiological growths that are occurring within the structure (e.g. nodule, plug layer, encrustation, tubercle, and rusticle): methods are defined below for each of the sample types; diluant is sterile distilled water (SDW) but in the event of high salt contents in the sample then SDW can changed to either sterile (by autoclaving) 4% or 12% seawater salt solution in distilled water with solution being selected by sample salt concentration (2 to 8% would use 4%; >8% would use 12%); correction factor is the multiplication of the predicted active cell population generated by the various DBI software assuming that there has been a 15ml liquid sample employed and the correction allows the

projection of the population per ml or per gram in the original sample (as pac/ml or pac/g respectively); note that pac can be directly converted to colony forming units (cfu) if required for comparative or regulatory purposes.

There are four methodologies (A, B, C and D) defined in Table 5.1.1 which require a different procedure that the standard defined in the "Certificate of Analysis" which accompanies each box of Bart testers. The changes are defined below:

A. This method is specifically for liquid samples that have poor clarity (i.e. are cloudy) and could not be used at 15ml volume. Here the procedure involving these types of samples would be unscrewing the cap of the inner tester (placing it on a clean surface without turning it over), and adding the defined volume of sample (e.g. 1.5 or 0.15ml) directly over the ball resting in the base of tester. It should be noted that this assumes that there are no particles in the sample that are greater than 0.1mm. If there are larger particles than that in the sample then there is a risk of ball jam (where the particles collect around the equator of the ball and prevent it from rising). If this is a probability (i.e. ball jam risk) then revert to method B. Once the liquid sample has been added successfully with no ball jam evident, then add the SDW to bring the liquid up to the fill line (13.5 or 14.85ml). The ball will float up as the SDW is added. It should be noted that particularly in the case of black waters there would likely be a diffusion front formed as the black water moves up into the SDW column in the tester. **Do not shake the tester** but allow the process of natural diffusion to occur. In some cases the sample immediately after charging to be very turbid, dark and not being suitable for detecting the reactions and activities. When using the VBR I or II systems it has been found that been found that these interferences abate and then the typical reactions can be read. For these cases set the VBR software for the time that the tester was started and then do not record any positive reactions / activities until after the tester has clarified. The use of sterile distilled water has been found in practise to be the most suitable diluant to allow the consistent clarification of the culturable sample solution in the tester.

B. This method can be employed for lighter soils and concretions. Here there is a high probability of particles leading to ball jamming and so the ball has to be removed prior to adding the sample. The sequence for adding the sample is as follows: (1) unscrew inner tester cap and place it upside down on clean dry surface;

(2) roll the sterile ball out into the sterile inner surfaces of the upside down cap;

(3) weigh out sample and deliver to the base of the tester using a sterile or flamed spatula;

(4) Dispense SDW as the diluant as specified in Table 5.1.1.;

(5) Lift the cap containing the ball and gently roll the ball back into the tester; and (6) screw back the inner tester cap.

Do not shake or agitate but allow the sample to gradually equilibrate with the diluant.

C. Sticky clays and clay rich loams create different challenges to testing in that the sample can disperse causing clouding and frequently causing ball jams. For these reasons the method employs only 0.1g of sample and the method followed is the same as for B above except that stages (4) and (5) are reversed. Here therefore the ball is returned to the tester before the diluant is added. The reason for this reversal is that the ball being admitted before the diluant allows the diluant to flow around the ball and mix with the sample. This allows a more even dispersion of clay-based sample with a lower risk of ball jam.

D. Solid and semi-solid samples require the use of a sterile spatula. Stainless steel spatulas need to be sterilized before use and this can be done by steam sterilizing (autoclaving) within a sterile package; or the spatula can be sterilized by heat immediately before being used by moving in to and out of a Bunsen flame for five seconds. Make sure that that spatula does not get too hot to safely hold by the handle. For the HAB- and APB- testers, the protocols described above call for the tester to remain unshaken (undisturbed) during set up. This means that the application of the chemical (methylene blue for HAB-, and bromocresol purple for APB-) in the cap has to be dissolved and mixed into the body of the tester without shaking. This can be achieved by pippetting 1.0ml of sterile distilled water into the upturned inner tester cap inside the circular flange. The chemical (methylene blue or bromocresol purple) is dried into the cap and takes one minute to dissolve as a blue or purple solution respectively. While the chemical in the cap is dissolving then the tester could be set up as per standards described above except that 1.0ml less of diluant would be added. This would be because that one ml volume would be added with the dissolved chemical in the cap. Once the tester has been prepared (without the cap) then the contents of the cap are poured over the floating ball in the tester and then capped. Do not agitate, shake or otherwise disturb the tester once capped and racked for observation. It will take approximately one hour for the chemical to diffuse into the sample liquid column and

generate an even blue or purple color.

There is one other condition for the HAB- tester under which it is important to dissolve the methylene blue in the cap rather than follow standard protocols. This is when the sample contains greater than 1% total salts or >100ppm of total petroleum hydrocarbons (TPH). When applying the standard protocol these constraints may cause may cause the methylene blue to change to a green color or even bleach completely. By pre-dissolving of the methylene blue chemical within the cap using sterile distilled water then the chemical goes into the solution phase to become blue before it comes into contact with the sample to be tested. When dissolved in this manner there is no impact caused by the salts or TPH when exposed to the salts or chemicals in the sample. In the APB- tester there is an additional need to ensure that the sample is not acidic (pH, <5.5) since this would cause a premature detection of a positive. Where the soil or water sample has a pH of <6.5 then it is recommended that the pH in the sample (if water) be corrected using sterile one normal sodium hydroxide to 7.1±0.1. If the sample is solid or semi-solid and is acidic then the sample would still require adjustment to 7.1 and this should be done to the original sample just prior beginning the full APB- test.