Chapter 2

Characteristics of the BART tester

2.1 Physical Characteristics

In simple terms the Bart tester contains a floating ball which throttles the movement of oxygen down into the liquid sample beneath. This creates oxidative (oxygen rich) conditions just under the ball and reductive (oxygen free) environments at the bottom of the tester. At the same time the dried chemical pellet begins to dissolve releasing selected nutrients into the sample being tested. Thus initially there is oxygen rich environment over an oxygen depleted environment along with chemical nutrient fronts moving up into the sample under test. For the bacterial communities in the sample a diverse dynamic micro-environments and beggars can be choosers! The key to finding different bacterial communities lies in the selective nature of the dried chemical (nutrient) pellet.

Weights for the different Bart testers is included in Table 2.1 and give the weight of the lab Bart separately to that of the field Bart. Minor differences in weight are due to the differences in the selective chemical (nutrient) pellet.

Table 2.1 Weight of lab and field Bart testers

Bart type	Weight lab tester	Weight of field tester	Weight of reaction cap
SRB-	16.23	37.70	NA
IRB-	16.23	37.70	NA
SLYM-	16.57	38.04	NA
DN-	16.40	37.87	NA
N-	22.58	44.03	2.38
FLOR-	16.58	38.05	NA
ALGE-	16.15	37.62	NA
POOL-	15.85	37.32	NA
ENH-	16.57	NA	NA

Note: weights are in grams and relate to unlabelled testers; normal variance is ±1%; and NA indicates “not applicable”.

Both of the tubes (inner and outer) tubes used in the production of the Bart testers are primarily constructed of medical grade polystyrene. Table 2.2 gives the basic dimensions for these tubes.

Table 2.2 Dimensions (mm) and thread size for cap for inner and outer Bart tubes

Item	Inner	Outer
Thread size for cap	28 - 400	38 – 400
Tube overall height, mm	89	96
Average wall thickness, mm	1.75	1.75
Mid-Point ID, mm	21	31.5
Mid-point OD, mm	24.5	35

The prime feature of the tester is that it utilizes a 15±0.2mL of whole liquid or diluted turbid, semi-solid or solid sample (see also Table 5.1.1). One unique feature of the tester is a floatable Bart ball that has a diameter of 19.7±0.1mm and a density of 1.08±0.01 and hence floats in non-saline water with 80±2% of the ball submerged. For the inner tube of the Bart tester the inner walls are sloped upwards slightly outwards and at the floatation point for the charged tube where the Bart ball would float inside the inside diameter of 22.1±0.1mm meaning that there is a gap between the floating ball and the wall of the inner tube that averages 1.2±0.02mm when the ball is centrally positioned. It is this floating ball that acts as a throttle restricting the movement of headspace gases (e.g. oxygen) down into the charged water sample therefore creating a top down oxidation to reduction gradient.

There are two zones created by the floating Bart ball:

(1) Liquid above the equator of the ball and below the surface of the ball which is directly exposed to the headspace air with a volume of 0.33±0.02mL, and a surface area of 2.2±0.2cm^2 with an aspect ratio (surface area: volume) of 1: 0.14 indicating that conditions would be very oxidative; and

(2) Liquid below the equator of the ball has a volume of 12.8±0.2mL with the "surface area" at the equator interposed with the oxidative zone above was 0.788±0.02cm^2 which gives an aspect ratio (based on volumes above and below equator) of 1: 18.4±0.2. Such a ratio would generate reductive conditions in the bottom of the water column since headspace oxygen would have to diffuse past the throat of the ball (i.e. at the equator of the ball which would be the closest point to the vertically sloped walls of the tube).

In summary the charged tester has two primary zones for incubation of the sample divided by the equator of the ball. 2.2% of the volume (0.33mL) of the liquid sample is set within an oxidative zone above the equator and the headspace air volume. The remaining liquid sample (85% or 12.8mL) is below the ball equator and so is more likely to become reductive as any oxygen present in the water is used for respiratory functions by the intrinsic flora. Metabolism below the ball moves rapidly to a reductive type involving fermentation. These reductive conditions are likely to first form in the base of the tester where the selective chemical nutrient pellet is dissolving and diffusing upwards into the water column. These events mean that the oxidative-reductive potential (ORP) interface slowly moves up the tester as the oxygen is utilized and often with intense bacterial activity around the interface.

One unique quality of the Bart tester is that it presents to the bacterial community in the sample with unique and dynamic (micro-) environments that develop as the ORP interface moves up from the reductive conditions in the base along with the dissolving nutrient front that is diffusing upwards at the same time. At the same time oxygen is diffusing downwards from the headspace above the ball. Thus the deeper regions in a bacteriologically active tester are going to become increasingly reductive. At the same time the selective chemical nutrient pellet is dissolving and diffusing upwards into the tester's water column. This causes a dynamic series of selective actions controlling the types of bacteria that would now become active. These events may therefore trigger focussed accumulations of bacteria as there is the site-specific degradation of the chemicals and to create an active biomass. This may be observed firstly as a gel-like deposit (e.g. IRB- tester), plate-like mobile growth (e.g. SLYM- tester) or simply the generation of reductive conditions (e.g. HAB- tester) or acidic conditions (e.g. APB- tester). The speed with which these populations are observed can be directly measured as the time lapse and the type of bacteria involved can be determined by the reactions concurrently recognised. It should be

remembered that Bart stands for bacteriological activity (generated by the time lapse) and reactions (generated by the recording of specific reactions) within the tester.

Each tester product type is governed primarily by the type of dried chemical nutrient pellet incorporated into the base of the inner tester and they may be used with water based liquid samples (15mL) or diluted from semi-solid, porous, solid, or emulsified samples. In these latter cases then dilution or dispersion in a sterile water base is necessary to reach the 15mL of liquid volume needed for the tester to function correctly.

All Field testers incorporate the inner tube which is balled, and has the selective chemical culture medium incorporated into the base of the inner tester as a sterile dried crystalline deposit (or pellet), and then capped. The weight of the inner tester (as the laboratory version) without the medium is 16.03±0.03g (without labelling). When the field version of the tester is prepared then this includes an inner tester which is identical to the laboratory version but also includes a second (outer) tube and cap to allow effective use of the tester under the more rigorous conditions of the field. These field units weight 36.12±0.03g without labelling and media.

To protect the testers (field or laboratory versions) from humidity which would otherwise cause the dehydrated media pellets to absorb water, expand and possibly become mobile gels, all testers are packed and stored in sealed 0.12mm thick aluminum foil pouches. Pouches are sealed using a heat sealer set at 300±10 ^{o}C with a three second compression time. Pouches sealed in this manner have been found to be moisture proof. Different pouches are routinely used (see Table 1.2.1. for packing formats). In the triple pouch there are three field testers (190 x 300mm, 7.3 x 11.5”). In the lab tester pouch there is room for five laboratory testers (220 x 160mm, 8.5 x 6.15”). Weight of aluminum foil used per laboratory tester is 1.760±0.005g. For the field testers there is additional aluminum foil employed which comes to 5.031±0.01g per tester. Boxes of either the laboratory or field versions are shipped in a common cardboard box that includes either fifteen (15) or twenty one or twenty five (21 or 25) laboratory testers in three or five aluminum pouches each containing five lab testers. For the field testers then three triple aluminum pouches are used to contain a total of nine (9) field testers all sealed separately. These boxes accommodate either testers and each box includes a “Certificate of Analysis” that includes the batch serial number that is generated through the ISO 9001:2000 certified procedures for

every particular batch of 700 to 900 Bart testers depending inventory demands. Some distributers label the outside of the box with the expiration date but all “Certificates of Analysis” includes the expiration date. Boxes should be kept in a cool dry environment until used. The shelf life has an expiry data set four years after the packaging of the testers into the sealed foil pouches. Quality management of the packaging through to boxing and warehouse storage follows the manufacture’s ISO 901:2000 compliant procedures. Testers are manufactured under sterile clean room conditions from injection of the plastic tubes through to sealing in the foil pouches. All procedures are monitored to ensure that the final product sealed into the aluminum pouch remains internally sterile. There are therefore two basic presentations of the Bart tester in boxed sets of nine testers for use in the field; and boxed sets of fifteen or twenty five for use in the laboratory. These are known respectively as “field” and “laboratory” testers. Field testers are designed to provide additional protection to the inner tester to allow use under the more rigorous conditions in the field.