Chapter 8

Tester Function

8.1. How the BART tester functions

Bart stands for “bacteriological activity reaction test” which is a patented trademarked (BART™) apparatus commonly referred to as a tester. It is used for conducting a single cultural test for the presence of selected bacterial communities. This chapter addresses the fundamental mechanisms governing the effectiveness of the tester. While the test employs a total sample at an optimized volume of 15ml of liquid, the tester has been critically engineered (see Chapter 2 for physical aspects and Chapter 3 for cultural aspects) to allow the culture and recognition of only the selected bacterial communities registered by their activities and reactions within the tester. In the critical engineering and design of the tester attention was paid particularly to the environments that were created when the tester was charged with a sample (see 4.2).

8.2. Environments created by the BART testers

Perhaps the most unique feature of the Bart tester is that it generates a range of lateral micro-environments ranging from very oxidative around the ball to extremely reductive in the base cone. These environments are dynamically affected by the diffusion along the vertical diffusion gradient created by the selective chemical nutrients moving up from the chemical nutrient pellet in the base of the tester. to background levels around the floating ball. Once the tester is charged with the sample then that dynamic process occurs in which multiple micro-environments are created from nutrient rich reductive types in the base and moving upwards towards the nutrient poor oxidative conditions at the top of the tester around the ball. Essentially, as the selective nutrient pellet dissolves, then the diffusion gradient moves up carrying reductive conditions with it. This diffusion front carries with it the oxidative-reductive interface where the bacterial activities frequently become concentrated and, in consequence, early activities and reactions are sometimes seen at those mid-points

For the bacteria in the 15ml sample charged into the tester there are a number of interactions that

can occur. There is initially a rapid reduction in the dissolved oxygen levels particularly in the base cone of the tester. These are caused primarily by the bacteria becoming stimulated by the diffusing nutrient front and utilize the oxygen far faster than it can diffuse down the tester. A reductive zone now forms once the oxygen has been consumed in the base cone of the tester. As the aerobic bacteria respire above the reduction zone at a rate faster than the oxygen can diffuse down from around the ball then the reduction front moves up. Thus there is a dynamic changing in the environments within the tester based upon the amount of bacterial activity consuming the oxygen, the manner in which chemicals diffuse out from the basal pellet generating reductive conditions when the oxygen is spent, and the interaction between the physico-chemical natures of the sample with the chemistry of the diffusing nutrient pellet. Essentially environments in the tester are in constant change if the active bacteria react with each other and the local conditions within the tester. Each type of tester therefore reflects in unique manners expressed as reactions and activities within the tester.

Fundamentally the basic ideas involved in this patented tester concept which are based on the generation of distinctive micro-environments include the following specific attributes (see Chapters 2 and 3 for more details):

- Enhancement of the Bart tester environment is achieved by the addition of a dried specific culture medium that allows the Bart tester to become supportive of the targeted consorm of microorganisms of interest that may be in the sample under examination.

- Selective culture of any bacterial consorm commonly happens in a staggered manner as the natural shifting of the environment within the tester occurs along with the products of that cultural activity now causes a gradual change in the dominant bacteria active within the consorm. This would create conditions where there is a bacterial mutuality which supplants competition between the members of the consorm.

- Activities and reactions observed in the tester would therefore be the direct result of the presence of a suitable active bacterial consorm that had not been suppressed by any restrictive factors present within the sample. These various activities would be a reflection of the upwardly cascading ability of the various bacterial communities now

become active and create activities and reactions during testing. The tester systems therefore involve dynamic states in which mutualism precedes restriction as various members of the communities rise to a dominant state. This would also involve the suppression of other members of the community. In simple terms there is an ongoing "war" between the various bacterial members of the communities for "growing space" and dominance within the tester.

- End points in the Bart tester are represented by detectable activities or reactions that occur after a period of time and are recognized as being significant (see Chapter 3) to the positive detection of the community that is being investigated. It is recognized that this end point would be influenced by a level of activity and reactions between the bacteria within the community. Time lapse, when generated, represents the mutualistic and antagonistic interactions between the active bacteria in the community during the incubation of the tester.

- Essentially the Bart tester provides unique abilities to detect the bacterial activity level within the communities present in the sample during incubation. It can be expected that bacteria will be moving through the phases of nutrient diffusion upwards (as the medium in the floor of the tester dissolves and rises), and interacting with the physical and chemical nature of diffusing matrices including oxygen moving downwards in the sample column. This would be followed by the creation of limitations (e.g., depletion of nutrients and oxygen and the build up of culturally restrictive end products).

- Precision and interpretation are based upon the time lapse generated by a recognized state within the tester environment (e.g., going reductive, shifting to a lower pH, generation of specific colors or structures within that environment). This interpretation represents the time delay (lapse) over which a complex microbial biomass achieves a given and recognizable state. The time lapse therefore does not directly interpret into a number of cells unless pure culture studies are undertaken. It is proposed therefore to consider that the time lapse reflects the status of the consorm under investigation with two proposed states:

ACTIVE is a condition in which the bacterial community immediately becomes active in the tester.

STRESSED is a condition in which the time lapse becomes delayed because the community had to pass through induction (adaptation) before it could become active.

From the investigations to-date it would appear that a time lapse of at least 2 days (48 hours or 172,800 seconds). In general practise this time lapse (delay) can differentiate between an active community (<48 hours) and one that is stressed (>48 hours).