Further Information

This book "*Standard Methods for Bart Testing, third edition"* is based on "*Standard Methods for BART Testing in Environmental Investigations of Microbiological Activities, second edition*" and has been based on scientific opinion while working for 53 years in the sphere of microbiological ecology (since 1959). As a result these standard methods are based not upon established scientific literature but upon practical forensic investigations that have been conducted. These investigations led to the development of the patented biological activity reaction test (BART™) which has been commercially available since 1990. These testers have been used primarily by engineers and consultants in the water, chemical, oil and gas industries where it has proven to be simple to apply while being sensitive to bacterial activities and precise. Over the last twenty years, there has been a steady stream of feedback from users. In consequence of this steady feedback, the testers have been improved and received ISO 9001-2000 certification in 2001 and remains compliant into 2013. Manufacture of the testers has been solely performed by Droycon Bioconcepts Inc (Regina, Canada).

There are references to work performed using the testers and these have been summarized in a series of books published since 1993 and covering various applications with the primary focus on groundwaters, biofouling and corrosion. These books are listed below followed by the (bracketed) the major focus in each publication:

Cullimore, D. R, (editor, 1987) International Symposium on Biofouled Aquifers: Prevention and Restoration. American Water Resources Association Technical Publication Series TPS-87-1, published by AWRC, Bethesda, Maryland (this was one of the first attempts to scientifically address the issue of biofouling in groundwaters and the methods for regenerating impacted water wells).

Cullimore, D.R. (1993) Practical Manual of Groundwater Microbiology. Lewis Publishing, Chelsea, Michigan, pp. 403. (this became a bestseller for the publishers and it

introduced the BART testers as major microbiological forensic tools. Copies of this as a pdf are available free of charge from a number of websites and deals primarily with the plugging of water wells)

Cullimore, Roy (1999) Microbiology of Well Biofouling, published by Lewis Publishers / CRC Press, Boca Raton, Florida. 435pages. (this book refined the methodologies that had been developed from 1986 to 1999 for the application of the testers primarily in water environments).

Alford, G. and Cullimore, D. R. (1999) The Applications of Heat and Chemicals in the Control of Biofouling Events in Wells, Monogram in the Sustainable Water Well Series (D. R. Cullimore, series editor) published by Lewis Publisher / CRC Press, Boca Raton, Florida 205 pages. (From 1988 through to 1999 there was a series of investigations of the ways in which BART testers could be used aid in the regeneration of biofouled wells in the USA. Many of these projects were developed with the support of the U.S. Army Corp of Engineers.)

Cullimore, D.R. (2000) Practical Atlas for Bacterial Identification published by Lewis Publishers / CRC Press, Boca Raton, Florida. (From 1966 there was seen to be a need to develop a more condensed approach to the classification of bacteria. This book was a best seller. It introduced two dimensional mapping and the tester technologies into the classification of bacteria).

Church, R. Warren, D. Cullimore, R. Johnston, L. Schroeder, W. Patterson, W. Shirley, T. Kilgour, M. Morris, N. and J. Moore (2007) A study of Living History, World War II Shipwrecks in the Gulf of Mexico. Artificial Reef Effect in Deep Water, U.S. Department of the Interior; Mineral Management Services, Gulf of Mexico OCS Region, New Orleans. 239 pp. published by PAST Foundation, Columbus, Ohio. (This book deals more with Deep Ocean and WWII steel shipwrecks but does touch on the microbiology and the BART testers. This book (ISBN978-0-9799909-3-9) should be in all school libraries as an introduction of Deep Ocean life and corrosion).

Cullimore, D.Roy. (2008) Practical Manual of Groundwater Microbiology, second edition. Lewis Publishing, Chelsea, Michigan, pp. 376. (From 1999 to 2008 there were a number of significant developments in the understanding of groundwater. The second edition was therefore published to improve the understanding of biofouling in groundwaters and how the BART testers could be used).

Cullimore, D.Roy. (2010) Practical Atlas for Bacterial Identification, second edition, published by CRC Press, Boca Raton, Florida, 327pp. (This book represented a major departure from the traditional Linnaean approaches developed as early as 1830. Here the premise is that all bacteria primarily function in community structures and their identification involves, in some case, the application of BART testers. This second edition should be appealing to those that have to contend with microbiological challenges in various environments).

There are numerous reports that have used the BART testers. Below is one of the longer assessments of water well rehabilitation techniques:

Jeffrey B. Lennox, CPG of Leggette, Brashears &
Graham, Inc (2006) AWWARF 91109 , "Application of Well Condition Assessment and Rehabilitation Techniques." Published by AWWA Research Foundation, Denver, CO. (This document was a detailed assessment of the various common practices applied in the groundwater industry for the rehabilitation of biofouled water wells mainly in eastern USA. BART testers were used through out to indicate the potential levels of bacterial challenges and effective regeneration of the treated wells).

The BART testers run under the patent listed below but has also been the subject of further patent applications and trademarks:

Cullimore, D.R. and Alford, G.A. (1990) Method and Apparatus Producing Analytic Culture, U.S. Patent number 4,906,566 (Concepts were developed in 1986 and the patent was accepted in 1990)

Cullimore, D.R. (2003) Floating Microbiological Growth Ball, U.S. Patent Pending

Notes

Individual reactions and interpretations were developed as the different testers were developed. Reactions were originally categorized numerically (1993) since there was some confusion over the color recognition particularly by observers having more limited color recognition. The movement to two letter coding was introduced to define reactions in 1999 and color comparisons were introduced using the standard Pantone® colors in 2010. Once major challenge with the colors is that every individual test that is considered positive may have slightly different shades of color. Each of the reactions in the testers have some relationship to physico-chemical processes that occur in connection with positive (bacteriologically active) tester. All of these reactions are well founded in chemistry with the exception of the formation of visible growths (as clouding, slimes, threads, floating plates and colloids). These are primarily the result of the growth of biomass within the tester either as dispersed or flocculated biocolloids, as defined structures such as sheaths (tubes) or floating plates.

Other reactions commonly represent a direct and indirect color shift resulting from interaction between the active bacteria and either the chemistry of the tester or sample itself. Essentially the selective chemistry in the tester is defined but the chemistry in the sample cannot be defined since this is dependent upon the environment from which the sample was taken. While reactions are established under defined conditions then these reactions can be affected by the chemistry of the sample being investigated. This means that the nature of the sample can affect the interpretation of the reaction by shifting the color spectrum being observed. This needs to be taken into account when interpreting reactions. Some of these interferences are described in the next paragraphs.

Iron in samples used in testers is possibly one of the major interference factors. This is because bacteria in the sample can shift iron relatively easily from the oxidized insoluble forms (ferric) dominated by yellow, red, brown color shifts; or to reduced soluble forms (ferrous) which usually are in the green color shifts. Iron-rich samples are most likely to interfere with reaction patterns particularly when the total Fe exceeds 3ppm. This does

not necessarily render the reaction unreadable (redundant) but does mean that there will be minor color variations. Examples of these interferences for different testers are described below. IRB- tester is affected by iron content in the sample and this is addressed during the four phased interpretation (3.1.2). If there are iron oxidizing or reducing bacteria in the sample along with a significant iron content then the first phase reaction will reflect this with GD (green diffusion) in the event of iron reduction dominating; and YD (yellow diffusion) dominating in the event of iron oxidizing bacterial activities occurring. Usually these two reactions are not taken as positive declarations for the IRB- tester but they do indicate the type of events that are likely to occur in phase two. Generally these two (GD and YD) reactions occur without clouding and are not considered as being directly linked to bacterial biomass activities. They should be viewed as precursors to second, third and fourth phases of the reactions. GD will usually lead to FO and on some occasions: GC, RC and BL whilst YD would move towards CL, BG, BC and BR and possibly a BL. It is when any of these secondary or tertiary phased reactions first occur then that tester is declared positive. It should be noted that while the FO reaction is a signal for reductive activities secondary reactions can involve iron oxidizing bacteria. Here the foam forms a ring around the ball and iron oxidizing bacteria can then grow on top of, or in the foam generating the typical ferric colors. It is not uncommon to see the foam generate orange to brown edges to the bubbles as the ferric forms from the activities of the iron oxidizing bacteria. On rare occasions the ferric product will form as an encrustation over the foam and also penetrate into it. For the IRB- tester there can be additional reactions where iron is significantly present in the sample. These relate to cases where there is a terminal black liquid (BL) forming as a tertiary reaction. This most commonly would involve the generation of black iron sulfides but it could also involve the generation of black iron carbonates (siderites). This is more likely to occur if there is an abundance of carbonates in the sample (generating a primary WB reaction). If there are high organics in the sample then there can be a black liquid (BL) reaction resulting from reduced organic carbon particulates.

Iron can also interfere with the HAB- tester in a minor manner. Here the iron from the sample reacts to create a yellow instead of clear solution when the methylene blue begins to bleach out (become reductive). In the SRB- tester there is already an abundance of iron

from the tester's pellet. This interacts with the hydrogen sulfide to form black iron sulfides. In the event that there is an excessively high level of iron in the sample then the culturing sample may generate yellow, orange or light brown colors. This is, however, not common. There has not been evidence of the iron content interfering significantly with the other testers.

One reaction that has caused particular concern is the BL (black liquid) reaction that occurs most commonly in the IRB- and SLYM- testers. Originally it was considered that this was caused by the generation of daughter products around iron sulfides or carbonates. However more recent researches into the BL reaction phenomenon have found that the BL reaction can occur in samples with low iron content. Here the BL forms as an ascending formation of fine black particulates that remain in suspension while forming from the base upwards. This reaction is particularly common in the SLYM- and HAB- testers. From analysis of the BL- positive testers it would appear that the black particulate suspensions triggered by reduced organic carbon compounds including elemental carbon. Conditions in these testers can become extremely reductive in the base that could trigger the stripping down of organics to hydrocarbons and carbon. Frequently these types of BL reactions also lead to the generation of gases which evolve from the tester. Analysis of entrapped gases has revealed the presence of methane and hydrogen and both of these can be the result of reductive biochemical activities involving the bacteria active in the tester.

Turbid (cloudy) water samples have been a perennial problem when attempting BART tests since they make it much more difficult to determine the points when a reaction would be declared. This has been addressed in 4.16. Here the only solution is to dilute the sample with sterile distilled. In the event that the sample has a high salt content (see 4.17) then the better diluant would be filtered (0.45microns) and sterilized (by autoclaving or steam sterilization) sample. This would mean that the bacteria would not be traumatized by shifts in the salt concentration. Generally where dilutions are necessary then the normal range is between one and three orders of magnitude. This would mean that the time lapses would be lengthened with the greater dilution. However the predicted population could then be corrected by multiplying the number by the dilution factor. For example testing performed at two orders of

magnitude dilution would mean the predicted population would have to be multiplied two orders of magnitude (i.e. x100).

Protocols are defined in the boxed testers and the "*Certificate of Analysis*" included with each box of testers and gives the standard method for water samples. This standard methods book includes protocols modified for soils, turbid waters and other environments. Chapter twelve specifically addresses some of the more unfamiliar environments where limited investigations have been undertaken. In the two editions of the "*Practical Atlas for Bacterial Identification*" some references are made to these environments. The second edition includes a partially tester-based identification of the bacterial consorms dominating different habitats. Essentially the whole process of recognizing bacteriologically influenced activities shifts from the Linnaean concepts (designed for plants and animals) to a more holistic approach. The bottom line is to know what the bacterial communities are doing within their habitats that influence both the local and regional environments.

In 2009 it was realised that the use of the adenosine triphosphate (ATP) had value in the rapid determination of the presence of active bacteria and this was developed as the enhanced total ATP test. This E-tATP test is able to determine bacterial activity in the picogram scale (10^{-12} grams/mL). While E-tATP does detect active bacterial cells it does not detect passive cells. When Bart testers are applied then the cultural conditions will also trigger activity in the dormant (passive, "sleeping") cells that can also then grow along with the E-tATP recognised active cells. The net outcome of this is that Bart testing will commonly record high levels of bacterial community activity in terms of cultured cell numbers than would be extrapolated by the E-tATP testing.

In the development of the visual Bart reader (VBR) system the objective was to increase precision and decrease operator time using the system. This is done using a rack that allow either 9 inners or 4 outers on each of two rows for a total of eight field testers or eighteen lab testers. These testers are illuminated from underneath. Time lapse photography is used (every fifteen minutes is the recommended sequence timing) using a camera that records the .jpg images onto a memory card. When the card is moved to a computer carrying the VBR or %CBR software then it is possible to interpret the images

to generate time lapses, populations and reaction pattern signatures. %CBR can only be used with the HAB- and BOD- testers and has the advantage the UP or DO reactions are determined by the software to generate time lapse and population predictions. VBR systems have another major advantage in that the operator does not have to come in to read the testers.

Limitations

These standard methods for Bart testing has been developed since the patenting of the methodology became pending in 1986. This whole process of development has been one of practicality to find which tester formulation appeared to be the most effective at the detection of potentially active bacterial communities. The philosophy revolves around the concept that bacteria function in communities ("lumps") rather than "split" into individual strains belonging to particular species within a genus. The latter is the dominant reductionist approach in microbiology and today the splitters far outnumber in every sense the lumpers except practicality. Splitters follow the traditional Linnaean dogma that all microbes have to be separated to genus, species and beyond with the belief that all bacteria need to be studied and identified at that level. This ignores two fundamental factors in microbiology: (1) that in the real environment bacteria commonly operate in communities containing anywhere from six to one hundred different types all living in harmony; and (2) attempts to culture "pure" cultures essentially means destroying the "pure" community and concentrating on only those that are able to be cultured. Today we are only just beginning to identify the significant bacterial communities and have distorted the basic understanding of bacteriology by concentrating only those that can be growth synthetically in scientifically reproducible manners. This book is basically for the lumpers who need to know where the bacterial communities are and what their functions are. This book therefore is the result of direct practical experience, discussions with many like-minded people who share the common need to know how bacterial communities affect their ability to effectively manage their bacteriologically influenced problems. May the Barts be with them!