Third Edition, 2013

This book “Standard Methods for the Bart testers” been published by Droycon Bioconcepts Inc. in 2013 as a general guide in the present practices of using the Bart testers to determine more effectively bacteriological activities DBI provides Manufacturing and Consulting Services for our Environment and specializes in the manufacture of the Bart testers. Bart means “biological activity reaction test” and is a patented technology (with patents pending) functioning under the trademark “BART”. These are commonly referred to as testers or Barts and come in two formats. For use in the field the tester comes in a double tube format which is more robust for use under field testing conditions. In the laboratory the tubes comes more economically packaged in a single tube format that is more convenient for lab use. These are referred to as the lab Barts. For the time lapse monitoring of the testers using the visual Bart reader systems (VBR) then the lab versions should be employed. The contents of this book have been presented to allow the users understanding of the functioning of the Bart testers and the manner in which they can be effectively applied.

This pdf version forms the third edition which was published in February, 2013 and can be downloaded with no charges from the opening page of www.dbi.ca. Previous editions (draft, first and second editions) are effectively replaced by this edition which contains the latest information in a standard format

Read, Understand and Enjoy

Questions and Answers always appreciated

Note that the contents list also forms an index to text and tables and is presented at the back of the book.

Foreword

In some ways the late George Alford (who played a major role in conceiving the Bart testers) could understand that this approach was very valuable when looking at the detection the "swarms" of nuisance bacteria that can grow and cause problems in all forms of liquid water. George thought that there needs to be a simple basic test that anyone could use reliably. Well as it happened I helped him with development of the blended chemical heat treatment (BCHT) in biofouling water wells and he then helped me with really making the Bart testers simple to use.

This edition of "Standard Methods of BART testing, third edition" has its foundations, indeed into the microbiology knowledge tree where it grows ever deeper and forever growing new roots of knowledge and understanding, Through trial and error, failures and learning, and the odd "Eureka!" the Bart test system was born at Droycon Bioconcepts Inc in Regina, Canada. The first Bart tester was made in 1986; the first sale was in 1990 with the beginning of manufacture. There has not been any attempt to grab the market but rather just let the sales grow (organically). This slow growth has been a Godsend since we have been hearing back from customers (thank you), making corrections, and improving the product. Today the BART tester is made in-house using clean rooms, automatic dispensers, plastic injection moulding for the tubes, and since 2001 full ISO 9001:2000 certification was obtained to make sure we are doing it right. Since then quality management has been used to assure product quality, the precision of claims, while retaining an open mind to improvements that are included in this edition. Major improvement include the introduction of ATP testing as the E-tATP, development of an alternate approach to the biochemical oxygen demand (BOD) test for wastewaters, identification of bacterial communities using the RASI-MIDI technologies, improved dispersants (e.g. CB-D) which have a superior ability to break up various forms of biomass, and the VBR I and II systems.

This edition has been documented to further standardize the methods (protocols). In general the Bart testers are seen to be simple to use in the field or the lab, and give precision without the need for deal of statistics, math or interpretation. This standard method book addresses the potential use of Bart testers that can unlock keys to bacterial activities in places where no agar plate has dared to successfully explore! At this time Bart testers have been used in deep ocean, sedimentary rocks, coals, oils, natural gases, sediments, slimes, scales and even in clouds! Of the

latter investigations on clouds then examining the falling rain using Bart testers reveals commonly ten to thirty million bacteria per mL with a narrow range of genera in the bacterial communities. The premise becomes that anywhere that you can find liquid water then you will also find bacteria. In these standard methods it has to be realised that bacteria in the big wide world of environments commonly operate as communities commonly containing between six and one hundred and twenty different genera and species. It is a dog eat dog (or is it microbe eat microbe) world out there and any non-performing species is eaten up by the rest of the community without hesitation unless they can hide! Obviously it is a world dominated by fittest while the others can chose to go to sleep (which is a very common practise in the bacterial world!).

Bart testers have become successful because they are both simple to use (for the operator) and comfortable for the bacteria whose activities are being investigated. They have been used on all of the continents and also in the oceans in between! There are many who now guide the direction of the Bart testers to achieve their goals of detecting specific bacteria with precision and speed. Detecting bacteria means that we are looking for the "lumps" of bacteria that form into coherent communities of need and purpose. In DBI we have a team of people who all share their enthusiasm to make the testers better. I would like to thank particularly: Natalie, ("and where's the money coming from for this?") Vincent (Webdude extraordinaire), Derek ("tweak, tweak!"), Myrna ("the inventory needs to be bigger"), Donna ("it's really shiny now"), and Ahmed ("yes it will be done by 4:30!). Thank you all for making the Bart team work. Beyond the horizon of Droycon towards the fourth edition (yes there will be one!) there are the many without whose help, thoughts, questions, suggestions, criticism and even answers without whose thoughts we could not be anywhere near where we are today. The list would exceed the number of pages in this book but I will just say thanks and keep it coming. Finally I would like to acknowledge the Prokaryotes for allowing me to play with them and maybe understand them a little better. Silence is the beginning and a void is the end but we must all remember that, while dwelling on Oceania (also known wrongly as the Earth), the biggest all-embracing biomass is formed by all of those bacteria that like to call Oceania home.

D. Roy Cullimore

Background

This document forms a compendium of the "*Standard Methods for the Bart testers, third edition"*. Bart testers have been used commercially for over 23 years by consultants, engineers and plant operators who wish to determine the nature of bacteriological activity that is affecting their results. While traditional microbiology laboratories can attempt some of these analyses the Bart testers have the advantage of being very user friendly generating results that are often directly applicable by finding the simplest answers to the questions or concerns. DBI grows because we listen, adapt and hopefully make better products and services.

The Bart tester comes in two basic forms for use in the field (**field** tester) and laboratory (**lab** tester). Here the field tester is more durable and hence more expensive while the lab tester is "bare bones" for use in a laboratory setting with trained technicians using racks or the VBR I or II systems. Both testers share the same basic premise:

(1) examine for the selected activities of the bacterial communities in the sample;

(2) set up inside the tester different environments from reductive-nutrient rich in the base to oxidative-nutrient poor in the top of the sample in the tester;

(3) use the sample undiluted as 15mL if liquid, and if solid then use between 0.15 and 1.5g of material with make up to 15mL (total) using a recommended diluant;

(4) under no circumstances should the tester be agitated or shaken except where included as a part of the protocol (see HAB- and APB- for examples of this);

(5) incubation (growing of the bacteria in the tester) is normally performed at room temperature (22 ± 2^{o}C) as a matter of convenience even though bacterial communities do not commonly grow fastest at this temperature;

(6) Different Bart testers are designed to specifically detect different communities of bacteria (called consorms); and

(7) all testers are sterile in their manufacture and the products are compliant with ISO 9001:2000 certification.

For the bacterial testing using the Bart testers are primarily to determine whether the sample possesses activities that relate to the detectable bacterial community. Here the activity is determined by the time lapse (length of time) that occurs before a reaction is recognized and recorded for interpretation. In the act of interpreting the time lapse into predicted active cells (or colony forming units) then different programmes can be used which utilize the same final formula. These programmes include "QuickPop" (functions only at $22\pm2^{o}C$ and allow time lapses in seconds, hours or days to be converted to populations; VBR software allows all of the data to be incorporated into an archived form which includes prediction of the population based on from the first recognized reaction that did occur; visual Bart reader (VBR I or II) systems that are fully automatic system employing a time lapse camera; and the confirmatory Bart reader (%CBR) software that specifically identifies positive UP and DO reactions in the HAB- tester and projects time lapse, predicted populations and the reaction type.

In the analytical use of the Bart tester the prime focus needs to be the recognition of the first activity or reaction that is recognized in the "Certificate of Analysis" for that Bart type for the prediction of the active population. This certificate is included with each box of Bart testers and includes basic information such as batch number and expiry date along with the reaction patterns that can be used to determine the time lapse. While subsequent reactions and activities are recognized only the first phase reactions are employed to calculate the population.

For the IRB- Bart tester the first reactions (phase 1) are not used since these relate to physico-chemical reactions between the tester and the sample and there are three further phases of reactions (phase 2, phase 3, and phase four) reactions which are used in the generation of a reaction pattern signature and prediction of the population (from the generated time lapse triggered by the reaction).

This document is separated into:

(1) primary and secondary objectives for using the Bart testers;

(2) physical characteristics significant to the functioning of the Bart tester;

(3) descriptions of the individual Bart testers that are in significant production in 2012;

(4) significant factors affecting the Bart testing practise;

(5) preparation of samples for Bart testing;

(6) disposal of completed Bart testers;

(7) specialty uses for the Bart testers;

(8) how the Bart tester functions;

(9) basic Bart parameter codes used to describe each of the testers;

(10) Bart conversion tables;

(11) Risk analysis;

(12) Innovative application of the biotesters in various environments:

(13) (13) Risk assessment for corrosion, plugging and health

(14) The use of the E-tATP to rapidly determine bacterial activities in samples;

(15) Applications for the visual Bart reader systems in the testing for bacterial activity;

(16) Interpretation of the periodic table to determine biological interactions.

This third edition includes additional materials considered important by some of the Bart users. The needs for ongoing attention to clarification and precision have been the most important reason for now releasing this improved and considerably more comprehensive third edition.

Chapter 1

Introduction

This chapter forms a summarized protocol for the use of all BART™ testers presently in commercial production by Droycon Bioconcepts Inc. This protocol therefore supersedes all previous methods and documentation appearing in print or on the web site, www.dbi.ca. Previous documentation remain valid descriptions of the testers and the methods recommended for analysis but this document as the third edition represents a more comprehensive description of the methodologies and the findings leading to the production of data and findings. This edition introduces the visual Bart reader systems (VBR I and II) and the development of a rapid biochemical oxygen demand (BOD) to replace the five day test methodologies. These improvements allow an initial fifteen minute biochemical test to determine low BOD samples followed by confirmatory cultural testing using the VBR system. Generated from this is the rapid BOD (rBOD) from the fifteen minutes test and predicted Rapid BOD (RBOD) for both the analysis for E-tATP in the sample the cultural testing using the VBR for the percentage confirmatory bacterial reductions.

1.1. Primary objective:

All Bart protocols use room temperature (22±1°C), unless otherwise recommended, for the incubation/ growth/ culture of the bacteria in the sample that are targeted by the environmental conditions presented in the Bart tester. It is recognized that the choice of this temperature is based on convenience since this is the normal room temperature range in offices, workshops and laboratories. It is recognized in setting that temperature as one of convenience it is not an ideal temperature for maximising the growth of bacteria (Cullimore, 2008, appendix J). Much faster growth is sometimes achieved using 28±1°C as the optimal incubation temperature but amazingly there can be so0me loss in precision (faster is not always better!). However the incubation temperature should reflect the environment from which the sample was taken. For example, 4±2°C, 12±1°C, 37±1°C, 45±1°C, and 54±1°C can all be useful temperatures and the maximum temperature for Bart testing is 80oC in a forced air incubator. Using 4±2°C can be very beneficial when examining very cold waters (e.g. arctic oceans, refrigerated foods, and melt waters. 12±1°C can be used as an optimal temperature for

samples that have come from a cool environment (e.g. refrigerator, shallow ground waters and oils). Warm blooded animals tend to have body temperatures of around $37\pm1^{\circ}C$ and this is a good temperatures to culture bacteria that are found growing under these conditions, Some disease associated bacteria often grow better at slightly higher temperatures and $45\pm1^{\circ}C$ can be effective for culturing these bacteria. There are occasions when an (industrial) environment heats the waters up to allow efficient heat exchangers (e.g. cooling towers) or improve the efficiency of dissolving salts (e.g. extraction mining of salts) and there higher temperatures such as $54\pm1^{\circ}C$ can be used.

1.2. Secondary objectives:

1.2.1. Bart testing methodologies can employ the video Bart reader systems as either VBR I or VBR II. In practise it has been found that the VBR systems can be most effectively used with two rows of nine testers (eighteen total maximum). Larger VBR units have been built commonly to monitor 60, 72 or 80 testers at the same time but there were significant operator challenges due to there being as many as four rows each holding up to twenty testers. Both the VBR I and II operate with eighteen testers (lab version) with illumination being upwards from beneath each tester row using daylight LED light panels. This analytical system utilises a time lapse camera (for recording .jpg images every fifteen minutes) and this data is recorded on an memory card for transferral to a computer with the appropriate VBR or %CBR software. This software now allows the determination of the time lapse, calculation of the predicted population and the reactions that have been observed.

VBR I system does not have any incubator and operates at the temperature of the room ($22\pm1^{\circ}C$). It is recommended that this temperature can be better regulated when an “intelligent” thermostat is employed to control both the heating and cooling as required. VBR I can be placed in a room with controlled temperatures of up to $45\pm1^{\circ}C$ but temperatures higher than that would require the VBR II. The difference between VBR I and VBR II is basically that the latter (II) version is built into an incubator that will control temperatures within $\pm1^{\circ}C$ up to a maximum of $62\pm1^{\circ}C$. Here the time lapse camera is not exposed to these higher temperatures and will function normally. For incubation temperatures below $22\pm1^{\circ}C$ the VBR II can be placed in a cooled room in which the room temperature is at least $4\pm1^{\circ}C$ below the required temperature that can be set for the incubator in the VBR II.

Time lapse camera can be used on either VBR system for the following Bart tester types: IRB-,

SRB-, SLYM-, HAB-, DN-, and APB- laboratory testers. Reactions are observable from the bottom-up illumination of the testers which allows coloured reactions to be observed along with clouding (turbidity).

1.2.2. **The recommended incubation temperature** for convenient testing using the VBR I system, or direct observation is room temperature (22±1 ^{o}C). It should be noted that both the QuickPop, VBR, and %CBR software systems allow the statistical prediction of the population size based solely on the first reaction-activity generated time lapse observed in the tester with the common temperatures being 22±1 ^{o}C and 28±1 ^{o}C. When other temperatures are selected then the standard software programs cannot be used to predict populations with precision. However data from these temperatures can be used to determine whether the target bacterial communities are present or absent and also, perhaps more importantly, the time lapses can be used to compare different samples. For example where biocides are being tested at a non-standard temperature then effective biocides would show much longer time lapses or no activity at all.

1.2.3. Bacterial populations are presented as predicted active cells per mL (pac/mL) in each ml of the fifteen ml employed in the sample. These predictions are calculated using specific equations generated for each of the Bart tester types to relate time lapses to the recognizable levels of specific bacterial activities. For each type of tester the formulae are generated by a combination of suitable cultural test methods commonly involving bacterial communities and selected mixed or pure cultures of bacteria. Most test methods employ the use of selective agar spread plate technologies which generate data as colony forming units (cfu). For the generated equations these data (cfu and time lapse) are used to create the pac/mL which may be considered for practical purposes to be equivalent to cfu/mL. Election of the pac/mL as a common term is based upon the premise that the Bart tester only reacts to the presence of specific communities of bacterial cells that are active in the sample. Chapter three describes the various BART testers and includes semi-quantitative populations for time lapses.

1.2.4. Bacterial communities are recognized the principal manner by which these bacterial communities function within the environment. They do not function as individual genera and species but do function together in an intelligent communal manner. It is these activities and

reactions that become detectable in the appropriate Bart testers. This detection leads from the occurrence of specific bacterial community activities within the tester generating a positive. This positive is achieved using the selective standard medium deposited as dried pellets in the cone shaped base of the tester. Reactions are recognized as being limited only to those standard reactions described in the "Certificate of Analysis" document that accompanies every box of testers. Activities are determined by the time lapse to the first recognized reaction achieved by visual daily observation (semi-quantitative) or using the VBR I or II systems. With the VBR systems the common time lapse interval between digital recording of the image is 15minutes. This frequency of recording allows the time lapse to be determined with precision and to quantitatively predict the population expressed in pac/mL or pac/g.

1.2.5. Sample clarity can be a problem that has to be recognized that every sample subjected to Bart analysis involves the generation of some unique color and clarity shift. Whether using direct visual observation (commonly daily) or the digital VBR systems the colors rendered as relating to a given activity are subject to variation. It should be noted that on some occasions the liquid sample may be very cloudy. This is particularly a problem with brines and waters with significant petroleum hydrocarbon contents. In these cases the two options are: (1) dilute the sample by ten- or one hundred- fold with sterile distilled water; or (2) use 1.5ml or 0,15mL of sample made up to 15mL standard volume added to the tester with sterile distilled water. Using deionised water is not recommended since while the water is chemically "clean" bacteriologically it could contain significant populations of bacteria that were fouling the treatment columns.

On occasions when the samples appear to be unreadable in the Bart tester (due to the intense clouding) commonly that clouding settles down within 12 to 24 hours and the tester become readable for the detection of activities and reactions. In the cases of clouding then using the VBR I or II systems the images taken (every fifteen minutes) for the first twenty four hours will show the gradual settling of the colloids and particles so that activities and reactions can then be observed. Here the start time should relate directly to the start of the testers monitoring without deduction of the time it took for the tester to clear. If it is considered important to use a compatible solution for dilution (e.g. brine) then this can be achieved by filtering through 0.45micron filters and then sterilising the filtrate. Here the diluant would now be a compatible

fluid and would dilute out the clouding with commonly a tenfold dilution. This issue is naturally one of trial and error and would vary from sample set to sample set. In the DBI laboratories it has been found that commonly the clouding will settle out and after the first day allow the normal monitoring for activities and reactions.

Variations in the activities and reactions generated by particular bacterial communities active within the sample would reflect the natural dynamics within that community responding to the conditions in the Bart tester. There is a natural variability in the form of these activities and reactions which expresses itself in various colors or clarity shift in the tester. To recognize this variability recognized activities and reactions are given in the form of two letters that best represents the observer's opinion of the reaction. There is no attempt to generate hard color matches but limit the determination to broadly based reactions and activities. If the reaction has been determined using the VBR I or II systems then that particular .jpg image of interest can be to DBI for further evaluation.

1.2.6. **Storage of the testers** are routinely in sealed foil pouches which have tear down tabs for breaking open the pouch. While sealed in the aluminum foil pouch there is extremely limited ability for water (moisture) to enter. When the testers are packed in the foil pouches there is a standard procedure to ensure that there are no nicks, tears or seal deformities that could cause water over time to enter into the pouch. Upon opening a box of Bart testers always be careful to ensure that these pouches do not get ripped or torn in any manner since this would cause moisture to enter the tester and shorten the shelf life. Evidence of moisture penetration becomes observable when the chemical pellet in the coned base of the tester becomes gel-like or liquefies and even begins move up (creep) up the walls of the tester or cause color changes. Note that the SRB during the normal production will generate some "creep" of chemical deposits up the first 4mm of the side walls of the Bart tester. This is a normal product of the manufacturing process and it becomes stable before the testers are sealed in pouches. Pouches are used to heat seal testers either individually or in groups of five or seven. The various combinations are shown in Table 1.2.1.

Once opened, the testers (or reaction caps) become exposed to moisture and there is a risk of compromising the data generated from the testers if they are not used immediately (i.e. less than 24 hours from opening the pouch). If for any reason the testers are not used immediately then

they can be stored in a cold dry environment for no longer than three weeks. Another alternative is to wrap the unused testers in 0.04mm heavy duty aluminum foil and adhesive (or duct) tape the edges to seal shut. Place in a cool dry place (e.g. refrigerator) and they should remain usable for three months. The foil pouches used by DBI have a thickness of 0.12mm and testers can remain useable for up to eight years but the recommended maximum storage time is four years from packaging.

Table 1.2.1 Tester combinations of Bart testers preserved in aluminum foil pouches

Pouch type	# testers	Tester type	Pouch #	# testers / box
A	1 (in 3)*	Field type	3	9
B	5	Lab	3	15
C	5	Lab	5	25
D	1	Field (+ 1 Reaction tube)**	8	7
E	7	Lab (7 + 1 Reaction tube)***	3	21

Note: *pouch type A includes three field testers sealed separately within a common pouch; ** reaction tube contains seven reaction caps); ***specific for the lab version of the N – Bart packaged as three pouches each including seven testers and one white tube of seven reaction caps.

It cannot be emphasised enough that even though the testers are sealed in 0.12mm foil, care should always be taken when handling the pouches to ensure that they are not punctured or torn in any manner since this would allow moisture to enter the tester. Compromise can also be caused by ripping the tear down on the pouches too violently and exposing more than one of the three field testers joined in a common set of pouches. Here the ripping could tear the foil of a neighbouring pouch exposing that tester to the risk of moisture entering that compromised neighbouring tester. Under no circumstances should the pouches be cut using scissors or casually ripped open since this would increase the probability of compromising neighbouring sealed pouches. Do not attempt to bag the unused testers in plastic bags or in sheets (e.g. cling wrap) since these do not effectively protect the contents of the tester from moisture. Once moisture has entered a tester then the selective nutrient pellet in the base of the tester can swell to a liquid gel and become mobile. This is one of the reasons for keeping the testers vertical during storage. If the testers are laid horizontally then the moisture will cause the media to move up the inside walls of the tester and even stick to the ball. It should be noted that the plastic spike sitting up from inside the cone through the chemical pellet perform the function of stopping the ball from

becoming stuck to the pellet.

1.2.7. Samples taken from low temperature environments (e.g. <18^{o}C) are likely to be supersaturated with oxygen leading to gas bubbles appearing quickly in the tester. If a tester is started using this cold water sample then gas bubbles will appear on the walls of the Bart tester and even under the ball. Allowing the sample to acclimatise to room temperature causes this gas to diffuse and not create gas bubbles or foam when the test is started. It is therefore strongly recommended that all cold samples be allowed to come up to room temperature for at least two hours (with four hours for optimal results) before proceeding with any testing using the testers.

1.2.8. Length of time for Bart testing is a critical concern. In other words "how long before you can consider the test to be negative?" Each tester has a set of unique cultural characteristics which means that it has a designed sensitivity to react to the selected bacterial community activity which varies from tester type to tester type. If it is necessary to select a standard testing (incubation) period then this was set at eight days at room temperature in 1999. However there are two major exceptions which are the N- tester that has a fixed incubation period of five days; and the ALGE- tester that can be incubated for as long as thirty days to ensure that even very low levels of algal activity are detected. The recommended time for the test incubation period has been set for the remaining testers at ten days, after which time the sample may be considered to not contain active of the selected type. Where negatives (absent) are found then these should be recorded as ND ("not detected") rather than as a cell number (e.g. 0 pac/mL). This more correctly informs the client requesting the test that no bacteria of the type selected for by the tester were found to be active. Generally after ten days it can be considered that a "not detected" would relate to less than 67active cells per Litre in the sample. One exception is the SRB- tester where late positives are sometimes detected at up to fifteen days after the start of testing. Where there is a specific interest on the part of the client in detecting sulfide production (primarily as hydrogen sulfide or black slimes/plugs) then it would be recommended that the SRB- tester could be extended to 15 days. In Waverley, Tennessee it was found that the testing extensions to 15 days allowed to early detection of sulfide producing bacteria before they significantly influenced the specific capacity / production of the water wells. In this case a preventative maintenance strategy was introduced which significantly extended the active producing life of

these wells. Here time lapse declines were used to predict populations and that this (shortening in time lapse) was used as the “red flag” marking the need for preventative or radical treatment.

Incubation periods at room temperature vary from one tester type to another. The most sensitive is the SLYM- tester that usually reaches detection of active populations in four days or less. It is the DN- tester that is the next most sensitive commonly detecting denitrification (foam gas production) in two to five days. The FLOR- tester while it detects bacterial activity as clouding in less than three days does not commonly detect fluorescence (PB or GY) until between two and six days and so the total time for the incubation of this test would normally be eight days. Heterotrophically active bacteria (HAB-) have a wide spectrum of incubation times from as short as three hours in primary influent sanitary wastewater to as long as six days. One challenge for the HAB- tester is the use of the redox indicator Methylene Blue which actives as a minor bacterial activity suppressor and, as a result, extends the time lapses by at least 40%. This is recognised in the various Bart software programs (Quick-Pop, VBR and %CBR). For the iron related bacteria detected using the IRB- tester there are four phases of reactions that can result in a ripple effect as one reaction is replaced by a second, then a third and even a fourth! Generally it is the IRB- tester that should be incubated for ten days even if one, two or even three reactions have already been observed.

If the client is concerned about the simple presence or absence of a given type of bacteria (e.g. IRB-, SLYM- or SRB-) then the ten day incubation period would be ideal since if the sample does not contain any significantly active bacteria then the result may be recorded as “absent” (or ND) in the case of no observable reaction occurring, or “present” if one of the recognized reactions had occurred.

Some clients require a population number usually as “colony forming units per mL” (cfu/mL). In the Bart tester systems developed over the last twenty five years the method being used differ fundamentally from the agar test methods. Perhaps the single major difference is the use in the Bart testers is the medium pellet dissolving to form the selective liquid medium in which bacteria can select and become most active in the most favourable environment. In the agar medium this selectivity is severely limited to the bacteria which are able to become active on an agar gel. The challenge for the bacteria here is that they have to use energy to “mine” water from that agar-based gel. As a consequence the Bart tester generates much greater opportunities for the selected bacterial communities to grow and generate recognizable reactions and activities rather than the

agar based techniques. The net effect of this is that the Bart testers do not generate countable colonies that can be counted but they do generate activities and reactions that can be measured through reactions or activities to generate the time lapse. It is the time lapse that now becomes the prime indicator of population size in bacterial communities active in the sample.

In the twenty five years of comparative studies it has been found that far more bacteria are detectable with a precision that is much better than for agar-based monitoring systems. Using the VBR system and triplicate tested of the sample commonly gives variances of less than 7% and frequently lower than 3%. Comparable sample testing using natural samples and comparable agar-based enumeration commonly gives population counts for the agar based methods commonly two to four orders of magnitude lower (i.e. hundred fold to ten thousand fold smaller) with less precise variance (as the percentage of the standard deviation over the average). Variances using agar-based systems rarely fall below 30% and commonly much higher than that. Agar enumerative techniques work relatively well when using cultures already adapted to the agar media (<5% of the projected total range of bacteria). Agar became established as the standard for microbiological investigation of pathogenic bacteria that were able to grow on these agar media. Here agar-based media were used to effectively isolate and culture the bacteria associated with typhoid, dysentery, anthrax in the late nineteenth century. This validated the role of agar as the prime culturing agent for bacteria. Today there is obvious evidence that agar media do pre-select only those types of bacteria that form colonies and be recognised. For those bacteria that do not grow on agar then their importance has been lost in the agar driven cultural dogma. The various Bart testers do allow these bacteria to grow and flourish by providing a whole smorgasbord of environments for them to choose from!

In the development of quality control strategies for the Bart testers it was found that using agar-compliant pure cultures of bacteria would help to generate comparative data for the development of population counts to effectively compare with the time lapses generated using the Bart testers. Since these statistical relationships between the time lapses could be linked to cfu/mL it was decided to use predicted active cells per mL (pac/mL) to compute the populations generated from the Bart testers. There is therefore a direct link between populations generated as pac/mL and cfu/mL with the caveat being that the agar spreadplate methods are inferior for the projection the true populations of active bacterial communities within natural samples.