Plugging and Corrosion Risks in Water Wells of all types

Risk Assessment Biofouling (RAB) Protocols

P0808 Water Well Rehabilitation

Determination of the Plugging and Corrosion Risks in Water Wells of all types

Risk Assessment Biofouling (RAB) Protocols for Water Wells

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Executive Summary

Injection and extraction wells form a major component in the disposal and production of water for all purposes. These well are set into the geology in such a way that the groundwater becomes the receiver or source of the water for the designed operation. It is inevitable that these wells will become subject a series of microbiologically influenced events that will affect the functional efficiency of the wells in service. This protocol uses three of the BART testers to monitor for the occurrences of: (1) plugging causing losses in specific capacity and deteriorating water quality; (2) changes in the pH of the water due primarily to reductive fermentative activities by bacteria; and (3) corrosion often leading to site-specific perforations of metal, or weakening in the strength of the metals. The protocol involves the development of a PAP (plugging, acidulolysis and perforation) risk determination that can be applied on a routine basis (e.g. every two months) to any well system presenting a potential concern.

Additionally some attention needs to be paid to the potential for water wells to be failing as a result of long term bacteriologically influenced events in and around the well. Any water well forms an intrusion into the natural environment that changes key critical factors in favor of bacteriological events leading to increases biomass which then affects the well's performance both quantitatively (through specific capacity shifts) and qualitatively (as the biomass shifts from a natural filter to a occlusive dam). When the bacterial activities begin to affect a well then the symptoms generally are (in order): increasing bacterial activity; declining specific capacity, failing water quality and finally failure of the well to deliver acceptable water in adequate amounts. By using the PAP testing protocol it becomes possible to regenerate the well to a more acceptable performance through preventative maintenance or radical rehabilitation. There is a potential for some bacteria to create a potential health risks. Three bacterial genera are recognized as being potential opportunistic pathogens via the produced water.

1 Introduction

Water wells whether vertically or horizontally deployed for the primary vehicle through which water in removed (extracted), or injected into ground water system. In these systems the water flow is either into, or out of, the well. This means that the water moves between a low surface area: volume (SA: V) ratio in the well that is often oxidative to a high SA: V ratio in the surrounding porous or fractured media that gradually becomes more reductive further from the well. Within and around the well are two distinct environments within which biofouling can occur differently. A further environment factor is created by the physical movement of water moving the water through the system. Here the major effect would be one of transient compression and turbulence. While the first reaction to such a system in operation would be that it could not be subjected to much bacteriological activity, the presence of two distinct environments (oxidative low SA: R ; and reductive high SA: V) means that that there is a high probability of biomass interaction at the oxidative – reductive interfaces (redox fronts) where these occurs [12]. Generally in groundwater situations about 80% of the biomass concentrates at the redox front. In water well systems it may therefore be expected that similar microbiological growth concentrations occur at these redox fronts [21].

The net effects of this biomass concentrating at the redox front can be summarised as causing the generation of biomass on the oxidative side, and the generation of corrosive/fermentative activities on the reductive side of the front [8]. These effects can materially impact the efficiency of the extraction or injection well. Biomass growth on the oxidative side could lead to plugging in or around water wells [23]. Plugging will reduce the ability of the wells to transmit water through the generation of biofilms on the surfaces (reducing the conductive area of the pipe) and through the interception of flow with a biomass that then prevents any free flow along the impacted section of the pipe. Microbiologically influence corrosion (MIC) commonly is associated with reductive conditions at, or close to, the pipe walls. There are two recognized MIC events that are common in these reductive conditions: (1) generation of hydrogen sulphide by sulfate reducing bacteria (SRB) [11] commonly leading to a radical focussed pitting of the steel;

and (2) generation of acidic conditions primarily by acid producing fermenting bacteria (APB) that lowers the pH into the range of 3.0 to 5.0 where lateral corrosion of the steel can now occur.

Most of the studies conducted on MIC linked steel corrosion are confidential to clients and so cannot be discussed. However researches on the steels present on the *RMS Titanic* are being subjected to both pitting and lateral generalized corrosion [6]. Both through consulting and the studies of sunken steel fabricated ship wrecks it has been found that the pitting form of perforation is more commonly associated with the SRB while the lateral forms flaking types of corrosion is associated with the APB [18]. Fundamentally the variety of biofouling that can occur can be considered to closely parallel the types of microbial activity causing plugging, clogging, corrosion and losses in water quality in water wells [9, 22, 23]. Essentially the critical connections between biofouling and the well systems relate to interactions between metal, plastic and natural surfaces and the ground water. Extensive researches have been conducted on sunken steel fabricated ships from the *RMS Titanic*[15, 16,19,20,28] and in the Gulf of Mexico, Mediterranean Sea and North Atlantic[33]. In all of those cases steel test platforms where placed on the wrecks and the rates of corrosion and biomass generation are being monitored. These studies led to the development of a new concept of the manner in which bacteria function at the water: steel interfaces using the *RMS Titanic* information as the prime example [7].

2 Biofouling of Metal Alloys and Plastics in Water Wells

From research on the biofouling of steels it has become evident that the bacteriologically influenced biomass does incorporate a number of distinct communities that appear as unique and definable structures [4]. While it has been accepted since the 1980s that biofilms were commonly formed on surfaces including steels, the role of bacteria in the formation of ferric rich deposits was not clearly understood until a basic definition of iron related bacteria was developed in 1978 [27]. From these various studies it became clear that oxidative conditions tended to create orange-red to brown ferric rich biomass in the presence of iron while reductive conditions created grey and black iron sulfide rich slimes

and encrustations. This was confirmed in the laboratory using mesocosms32 and in the field[30, 31]. From these studies it was determined that bacteria were intimately involved in the formations of slimes, nodules, tubercles, encrustations, floating biocolloidal particulates as well as the generation of gases. Various groups of bacteria were found to be precursors to the occurrence of MIC plugging and microbiologically influenced fouling (M.I.F.). This led in part to the development of a new bacterial classification system[10] in which the bacterial genera were displayed within a two dimensional atlas.

3 BART testing, history

Directions for investigations were focussed towards the rehabilitation of biofouling water wells[13, 29] and the development of suitable bacteriological testing systems that would declare not only the activity levels for specific bacteria but also the major communities present. This was addressed in 1986 with the development and subsequent patenting of the biological activity reaction test (BART™) and reported as being effective in 1990[21, 24]. Initially the BART testers were used to determine the risk of biofouling in water wells and the impact of rehabilitative treatments on the production characteristics of the well[1, 2, and 3]. In 2000 the potential to use BART testers in novel manners for the detection of other biofouling-related problems in water was addressed[17]. Here claims were developed around the clear advantages of the BART testers over the more conventional test methods that primarily utilised agar culture media that proved to restrict the bacteria recognized to only those that would form colonies or generate distinctive colour reactions on, or within, the agar. Comparison of BART testers was described in 2005[e] with the various other standard microbiological methods.

Concepts used in the BART tester that allowed it to be very sensitive and accurate was examined [f] and the major advantages primarily relating to the generation of an oxidation-reduction gradient during the test, the gradual diffusion of the selective culture media into the liquid phase and the utilization of a floating BART ball to stabilise the lateral environments within the sample being tested. Of all of the factors one of the most important is the composition of the selective cultural medium that diffuses up the BART

during test. This is described [g] and allows the effective investigation of various bacterial communities within a common water sample. Comparisons between time lapse and reaction data for the HAB-BART system was compared to the colony forming units for three strains of ATCC bacteria and good statistical correlations were reported[5].

Manufacture of the BART testers began in 1990 and since that time 1.7 million BART testers have been sold primarily into the water industry with secondary sales in the chemical, gas and oil industries. In 2001 Droycon Bioconcepts Inc became registered as an ISO 9001:2000 certified company and all stages of the manufacture were included to assure effective quality management. General information on the BART testers is periodically updated and the last update was in 2006 with the advisory DBIMANG06 providing general information. Three BART testers have been through the Canadian Environmental Technology Verification in 2002 for the IRB-BART [m] and the SRB-BART [q]. In 2004 the HAB-BART system was also taken through and approved using the Environmental Technology Verification [k] and the system was described [j] which included the first electronic reading system for the reactions. Critical in the establishment of precision is the interpretation of the time lapse (in seconds, hours or days) to the recognition of a reaction. This gives a direct evaluation of the population primarily as predicted active cells per ml and equivalent to the traditional colony forming units. These relationships were updated in 2006 for the bulk of the BART testers and the error incorporated when only daily readings were performed [n]. Over the last five years there has been developed a BART-SOFT program now in version 6.08 [f] which allows the automatic interpretation and archiving of entered data for later evaluation using Excel spread sheets and .rtf files formats.

4 Standard Protocols

Standard protocols exist for all of the BART tester products. Latest protocols for the BART testers that may be of interest to the fire sprinkler system maintenance are listed as: (1) APB-BART protocol DBASOP06; (2) DN-BART protocol DBDNSOP06; (3) HAB-BART protocol DBHSOP06; (4) IRB-BART protocol DBISOP06; (5) SLYM-

BART protocol DBLSOP06; and SRB-BART protocol DBSSOP06. These protocols commonly include various alternate scenarios to allow the evaluation of soils, encrustations, scales and various forms of solids.

5 Relationship of BART tester technologies to the monitoring of biofouling in water wells

In water wells there are a number of MIC events (leading particularly to the failure of some metal components and particularly steel fabrications) and MIF events that can impact the water flow through the system (plugging) and/or the water quality since the biomass acts as a differential biofilter causing accumulative and degradative functions to impact on the chemistry of the groundwater. Much of these microbiological activities are occurring within the attached biomass within the well's system and the water moving through the well. Often there could be no presence of the microorganisms in the water phase and yet they could be dominating in the attached phase. To compensate for the potentially low number of indicator organisms in the water the BART tester uses a 15ml standard sample size that is not diluted in the standard protocols. This therefore improves the sensitivity of the test to the detection of one active cell per 15ml sample (or 67cells per litre). Most standard microbiological techniques utilize fractions of one ml sample which would have the ability to detect down to 1,000cells per litre or one cell per ml. The BART tester offers a superior ability to detect the low numbers of active cells which might be present in groundwaters while most of the activity is occurring on attached biomass away from direct releases into the ground water. This one advantage of the BART tester is its greater sensitivity to the presence of low levels of activity. A second advantage is that, like the water well environment itself, the testers automatically generate a series of unique lateral environmental niches. This generation of later niches is created by the nature of the BART tester. Here the BART ball floating on the 15ml water sample restricts the entry of oxygen from the headspace air into the water column. This causes an oxidative environment to become restricted to just below the floating. At the same time any aerobic microbial activity within the sample removes any oxygen that was present in the sample. This cause the lower part of the BART tester to become reductive

(free of oxygen). Thus an oxidation (above) and reduction (below) gradient is built up within the tester along with a redox front (interface) where many microorganisms are optimally active.

Secondary niches are created as the dried selective chemical medium dissolve in the sample being tested and begin to form a diffusion front that moves up the tester. This means that for the microbes in the sample there are a number of selective environments created that have formed through interactions between the upwards moving selective chemical gradient and the stabilizing redox front between the oxidative zones (above) and reductive zones (below). It is a primary claim for the BART tester is that it is more sensitive to lower populations of the active and determinable microorganisms in the sample than traditional cultural or biochemical methods.

6 MIC and MIF events likely to occur in water well systems

MIC is defined as a microbiologically influenced corrosion while MIF is defined as a microbiologically influenced fouling. These two terms are very significant in the ongoing affective management of all types of water wells.

MIC may cause pitting leading to perforation of (particularly) steel or it can be associated with lateral losses to the structural integrity of the metals with associated increases in the porosity of those metal alloy walls. In general the pitting perforation phenomenon is considered to be primarily a result of the activity of sulfate reducing bacteria (SRB) that generate hydrogen sulfide from sulfate and it is this gas that causes an electrolytic form of corrosion. This form of corrosion will cause the steel in the wall to literally dissolve into soluble products leading to pitting and then perforation of the steel wall and loss of integrity (leaking). Hydrogen sulfide can also be generated by many proteolytic bacteria under reductive from organics including the sulfur amino acids. SRB generally dominate in waters that have a low organic content (e.g. < 5 mg/l TOC) while the proteolytic bacteria can become dominant when the water has a higher organic content (e.g. >10 mg/l

TOC). Pitting leading to perforation is likely to be as a result of the generation of hydrogen sulfide from one of these two groups of bacteria.

To detect the risks of pitting from these bacteria the SRB-BART tester can be used. Here the true SRB (generating H_2S from sulfates) are most likely to trigger a BB reaction (black base) in which the sulfide products are mostly accumulated on the floor of the BART tester as a jet black deposit. If the MIC leading to perforation involves both the SRB and proteolytic bacteria in the production of H_2S then the reaction pattern in the tester is more likely to be a BT reaction (black top) where the jet black sulfides form around the BART ball floating in the tester. While these reactions (BB, BT) are the first reactions observed in the SRB-BART tester a secondary reaction can occur as the H_2S continues to be produced and the whole of the contents of the tester turn black (BA, black all). Sometimes the BA can follow so quickly after the BB and BT reactions have been generated that these sequences can only be determined using time lapse photography of the type employed in the V-BART-READ system.

Pitting (confidential consulting project) which led to perforations of steel in gasoline storage tanks was found by experimental testing to range from 5.3±3.8mm/yr with an outlier generating 10.6mm/yr in a strong electrical field. Similar rates of perforation could be expected in sprinkler pipes under extreme test conditions.

Lateral forms of MIC causing a more generalised loss in the integrity of the metal walls leads to greater porosity in the steel and is generally caused by the acid producing bacteria (APB). Here conditions have to be low in sulfur organics and sulfate (otherwise the SRB are likely to dominate) and have sufficient organics to form a biomass over the metal surfaces. Once the biomass begins to grow then the reductive conditions will form within that biomass that would then allow fermentation to begin. Here the daughter products are commonly fatty acids that then cause the pH at the attachment surfaces to drop from 8.4±4 (in a typical functional biomass) to a pH range from 3.6 to 5.5. At these acidic pH values there can be a slow solublization of metal alloys including steels that then allows the bacterial biomass to penetrate through the fissures deeper into the metal

wall. This then allows the steel to begin to become more porous as acidulated water is able to move through these corroded channels into the steel. For a number of steel fabricated ship wrecks, steel test platforms have been placed by DBI for later recovery. For the *RMS Titanic*, four test platforms have been recovered and the rates of lateral embrittlement of the steel coupons have been assessed. Here the rate of losses of iron from the steel is at 0.03±0.01gFe/sq.cm/yr. These are extreme environmental conditions with high salt and pressures with low temperatures and limited available organics for growth.

Under the conditions of an active biomass attached to surfaces it has been found that commonly the APB dominate and the APB-BART tester has been effectively employed. Here the detection of APB activity is through the generation of acid (fatty acid) daughter products. These are detected by using a pH indicator that shifts from blue-purple to a dirty yellow color when acidic conditions are generated in the tester. Generally (like the SRB) the APB commonly will occur deeper down within the biomass and may not be detected unless the biomass is disrupted to move these bacteria into the water. It is more probable that the APB and SRB will be detected in water samples or samples of encrustations taken from impacted parts of the well where corrosive activities have been observed.

7 MIF events likely to occur in water well systems

The efficiency of a water well system can also be affected by any biomass forming within the casing, in the porous or fractured media surrounding the well, and most commonly on the slots or perforations in the screen. There are three principal effects: (1) biomass coating the inside casing of the well can cause reductions in the water quality of the produced water; (2) biomass forming within the slots or perforations of the screen may affect the rate of bulk transmission of water through the screen and/or cause diversion of groundwater around the biomass which could then change the quality and quantity of the product water; and (3) biomass growing outside the casing in the porous or fractured media could end up blocking the free flow of water (plugging).

The type of plugging –associated biomass can be very variable depending upon the chemistry of the groundwater (extraction wells) or on the interaction of the chemistries between the influent waters and the groundwaters (injection wells). These effect ts may be seen as scaling (high in carbonate); encrustations (high in ferric iron); nodules and carbuncles (capping high in ferric and contents high in organics); to slimes (high in bound water and organics). There are two bacterial groups most commonly associated with MIF events: iron related bacteria (IRB) that tend to dominate where ferric iron is accumulating (e.g. nodules, encrustations); and heterotrophic aerobic bacteria (HAB) that dominate particularly when the biomass is slime-like.

For MIF events the chemistry of the water is particularly important. Higher organics in the water (whether natural or added) are likely to stimulate the growth of HAB. High pH values are likely to increase the potential for scaling while iron in the water from the natural source are likely to stimulate ferric deposits within the biomass. These ferric deposits would then also be generated from the iron being released from the steel pipe once MIC events begin to occur. Two BART testers are commonly used to detect these events: HAB-BART tester for wells in which slimes and higher levels of total organic carbon are thought to be important factors in the plugging; and IRB-BART tester for ferric rich problems. These two tester types will be discussed separately.

HAB-BART tester is a simple test in which bacterial activity is detected by a blue color being bleached and replaced with a dirty clouded clear or yellow solution. This blue color may disappear from the bottom up indicating that the bacteria are active in an oxidative environment and more likely to be associated with aerobic activities such as plugging or slime formation and possibly scaling. If the blue color disappears from the top (just below the BART ball) down then this indicates a reductive environment and a greater probability for corrosion (MIC) to be occurring.

IRB-BART tester is more complex in the manner that it generates reactions. It does commonly as first reactions generate either clouding (CL, oxidative) or gassing leading to the formation of a foam ring around the BART ball (FO, reductive) and it can also detect

the risk of scaling. Generally these two reactions occur first and define whether the well is oxidative and dominated by aerobic bacteria (CL reaction); or the well is reductive and dominated by anaerobic bacteria (FO reaction). In the event that the two reactions both occur then the well may be considered to be transitory between oxidative and reductive and this is likely to present a greater level of concern since the redox front may be close to, or within, the water column of the well.

Another very early reaction occurs when the conical base of the IRB-BART tester turns white with carbonates. This commonly is generated during the first twelve hours of the test (WB, white base). At this time the WB is not recognised as a bacteriologically influenced reaction but it is used in the water well industry as a marker for the types of preventative maintenance and radical treatments that would need to be applied. Commonly when this applies then an acidic phase in the treatment is desirable to break down the carbonates that most commonly form within the encrusted growths. If acidic treatment fails then the types of carbonates may be such that a flip-flop between alkaline and acidic types of treatment may be necessary with the pH being manipulated at least 7 pH units. Alkaline conditions are normally generated in the pH range of 10.5 to 12.0 while acidic treatments need to extend down to 3 pH units (for organic acidic treatment) and less than 2 pH units (where inorganic acids are used).

Secondary reactions of significance to the biofouling of water well systems are: (1) generation of brown cloudy, BC, and/or brown ring, BR that indicates oxidative conditions with encrustations and ferric rich scaling probable; (2) occurrence of brown gels, BG in the lower third of the tester that indicates potentially reductive biomass that would support denser slime growth activities; (3) there is a terminal reaction in which the testers contents turn black (black all, BA). When the tester contents have turned black and this indicates that there is a greater potential for proteolytic bacteria to be actively generating H_2S and generating MIC. In summary, the occurrence of BC or BR indicates an iron-rich form of MIF which is oxidative; BG indicates that there is a denser biomass that is likely to generate plugging at the redox front under higher TOC conditions; and the

BA points to reductive conditions with higher TOC and a diverse bacteria causing the MIF.

Another BART tester that has value is the SLYM-BART tester which is the most sensitive of the tests to the vast array of bacteria able to use organics. Here the tester signals a positive from the generation of cloudiness and it usually is the first tester to react with a positive detection.

8 Recommended Detection Protocols for the detection of MIC and MIF events in water wells

Several key factors affect the recommended protocol. These will be dealt with separately below.

8.1 Temperature in groundwater systems is generally relatively stable with relatively little seasonal variations. In general groundwater is a reflection of latitude and can range from as low as $2\pm1^{o}C$ up to as high as $26\pm3^{o}C$ in continental North America. For convenience it is commonly recommended that all BART tests be performed at room temperature ($21\pm2^{o}C$) as the incubation temperature of convenience rather than at the optimal temperature for the groundwater source for the sample. Optimal incubation temperature common for the bacteria associated with biofouling water well systems in warmer environments is $28\pm1^{o}C$. Optimal temperature means creating the fastest culture-results.

Water well systems being operating on groundwaters at lower or even higher temperatures may require different incubation temperatures to the standard room temperature ($21\pm2^{o}C$). Ideally, incubation should be within $5C^{o}$ of the average temperature experienced for the groundwater during the four seasons. For example if groundwater was functioning normally over $3\pm2^{o}C$ then the optimal BART incubation temperatures would need to be within the range of -5 to $+5^{o}C$ of that temperature in order

to assure cultural detection of the biofouling bacteria using the BART testers. In this particular example possibly the ideal optimal temperature for testing would be in a refrigerator at 4±3^{o}C. Generally bacteria growing in cold environments (i.e. 0 to 15^{o}C) commonly have an optimal incubation temperature closer to the upper limit (12±2^{o}C) and so this is the temperature recommended for cold loving biofouling bacteria. Generally the lower limit for bacterial activity is considered to be -18^{o}C. At temperatures higher than 60^{o}C the polystyrenes used in the manufacture of the BART testers loose structural integrity and weaken. Recommended culture incubation temperatures are listed in section 8.2 under time lapse.

8.2 Time lapses are generated when a BART tester goes positive with a recognized reaction and is calculated as the length of the time period from the start of the test and the first moment that a positive reaction is detected. Commonly the time lapses are recorded in days for daily observations but hours or seconds can be employed where there is more frequent observations, a BART reader, or a V-BART-READ system is employed. Readers are tester specific and commonly generate time lapses to the nearest second. V-BART-READ employs routine digital image capturing that can then be played back to determine the time lapse for a recognized reaction. These time lapses are given in hours. Droycon Bioconcepts Inc have developed relationships between time lapse and populations for the predicted active bacterial cells per ml (pac/ml) for the major incubation temperatures of 12±2^{o}C (for cooler environments); 28±2^{o}C (for warmer environments); and 37±2^{o}C (for tropical and blood heat environments) as well as the standard 21±2^{o}C (room temperature).

Generally the length of time that a BART tester should be monitored before it may be considered negative for significant bacterial activity varies with the incubation temperature being used. For cooler environments (12±2^{o}C optimal or down to 4±3^{o}C in a refrigerator close to freezing) it is recommended that positive detection could still occur up to 20 days after the start of incubation. For warmer environments the temperature (28±2^{o}C) will usually yield any positive detection in 8 days after the start of incubation. For tropical and blood heat environments operating at 37±2^{o}C then any positive detection

should occur within 5 days after the start of incubation. For the standard 21±2^{o}C at room temperature it is normal for detection to be complete by 10 days for most BART testers but the SRB-BART which should be incubated for a further five days (to 15 days) in order to detect low levels of the more covert SRB.

8.3 Sample storage prior to BART testing, sample storage times prior to BART testing should be kept as short as possible since the bacteria within the sample are commonly in a state of shock as a result of the sampling process. It is suggested that these samples follow the common protocol developed as Appendices H to K in the second edition of the Practical Manual of Groundwater Microbiology [12]. This would mean that the sample would be kept discrete, protected from evaporation, and cooled down to 4±3^{o}C if storage is to exceed for hours. For shorter times then the sample may be kept as close as possible to the original ambient temperature of the sample.

9 Validation of Sample

This is actually a serious challenge since frequently the water from water well systems may be relatively convenient to extract as the sample but it may contain a non-representative number of the bacteria rather than those being targeted. This would be because most of the bacterial biomass within water well systems forms only parts of generally attached communities. Samples may not necessarily include representatives of the attached biomass that may or may not be present in the flowing waters moving through the well. Endoscopic examination using a down hole video camera will physically show whether there is defined forms of biomass attached to the walls of the well but not pictures of any biomass growing beyond the slots or perforations of the screen. Growths may commonly take the form of biofilms, nodules, tubercles, encrustations, scaling; or the biomass may be observed primarily as floating in the water (as particulates popularly referred to as “snots” or “floating slimes”). If the growth

creates a dam of biomass product in the water that then these growths can plugs up (restricts or prevents flow) the wells.

Floating particulates within the water are probably the easiest to recover during sampling with the other forms of biomass becoming progressively more difficult based upon fragility and structural integrity, form of the surfaces, density of the bacterial populations within the biomass. In cases where it is desired to determine the nature of any pitting leading to corrosive perforation of the pipe, then it is important to take samples that would include the biomass that is supporting the corrosion process. This may be achieved by changing the environment in the pipe to be inspected. Commonly changing the environment in a radical manner (e.g. turning off the recirculation of the water for an extended period of time; applying a low level of non-toxic detergent or penetrant, taking the temperature of the pipe to be inspected up, or down, by at least 10^{o}C). In all of these examples it can be expected that the bacteria active within the biomass will become traumatised and it is a common experienced that bacteria under these types of stresses will move out of the biomass into the water and then recovered with the sample.

10 BART Evaluations of Biofouling Risks to Water Well Systems, Risk Assessment Biofouling

The BART tester offers a simple set of tools for monitoring and diagnosing the risk of corrosion events in water well systems. In using the BART testers to assess biofouling risk there is a clear differentiation between those testers that detect MIC and those detecting MIF. For monitoring corrosion (MIC) risk there are two types of BART testers that have a role to play. In summary these are the SRB-BART (for the detection of pitting and perforation in steel); APB-BART (for the detection of more diffuse forms of lateral corrosion that can lead to the pipe becoming weaker and more porous). For plugging (MIF) risks it is the HAB-BART (for the detection of biomass size and activities linking to plugging) that often dominates where there is a high TOC content in the groundwater. If there is a significant level of iron in the groundwater (e.g. >0.1 mgFe/l) then there is a possibility that the MIF event will include iron related bacterial activities commonly

generating ferric-rich growths under oxidative conditions. Here the IRB-BART can be used to detect this type of biomass activity.

Each of these four recommended BART testers generate in the case of a positive, both a time lapse (commonly in days) and a reaction signature (commonly as two letter acronyms). Performance risks to the water well systems can be generated from this data. Time lapse gives an indication of the level of activity that was detected in a sample. In this case the shorter the time lag then the more active the bacteria are recovered in the sample. For the reaction signature (limited to one or two reaction types in the recommended BART testers, this can be used to determine the nature of the corrosion MIC risk directly (in the case of the SRB- and APB- BART) and of plugging MIF risk due to biomass generation (in the case of the HAB-BART and the IRB-BART). In most cases it would be expected that incubation would be at room temperature ($21\pm2^{o}C$) and the risk assessment below is established with that in mind. Because of the nature of the four recommended BART testers, the diagnosis of both MIC and MIF risks are taken on the basis of the time lapse and reaction signatures observed for each BART tester separately.

11 SRB-BART testers to detect MIC

This tester may function for as long as 15 days with two possible reactions (BB, BT) defined above. Table SRB.1 lists the relative corrosion MIC risk using the time lapse (days) and reaction signature. Risk is generated from this table.

Table SRB-1, Risk Analysis Biofouling (MIC) based upon time lapse and reaction type

	Day 1	Day 2	Day 3	Day 4	Days 5 -8	Days 9-10	Days 11-15
BB	9	8	8	7	5	3	1
BT	9	9	9	6	4	2	1

Notes: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown as being either of the BB (black base) or BT (black top) forms; risk numbers are shown for each cell in the table but the risk relates to a different forms of MIC corrosion; BB risks are defined to relate to a rapid perforation of the metal walls by H_2S generated by the SRB principally from sulfates; BT may also be related to the more generalised perforation of the metal walls but with the H_2S being generated proteolytic bacteria; MIC corrosion risk analysis is shown in each cell as a single digit from 1 to 9 depending upon the (ascending) severity of the corrosion; only one digit may be used for the calculation of the corrosion risk with priority being given to the reaction that is first observed; In the event of both reactions (BB and BT) being observed then the risk assessment is based upon the first reaction observed. In the event that both reactions are observed on the same day then priority is given to the BB reaction for calculating the corrosion risk.

Calculation of the corrosion risk for pitting-induced perforation (PIP) of the metal surfaces would consist of a single digit based upon the data generated from table SRB-1. In the event of a BB reaction it may be expected that there would be a relatively low biomass involvement and the pitting would lead rapidly to perforation. Where BT

reaction was first observed then the type of pitting would be likely more extensive in association with the greater biomass involved in this type of event. Perforation would remain the most probable outcome but the biomass may act to restrict water leaking through the hole in the metal or a gradual collapse in the metallic structures.

12 APB-BART testers to detect MIC

This tester may function for as long as 10 days with one possible reaction (DY, dirty yellow) defined above. Table APB-1 lists the relative corrosion risk using the time lapse (days) to the reaction signature (DY). Risk is generated from this table.

Table APB-1, Risk Analysis Biofouling (MIC) based upon time lapse and reaction type

	Day 1	Day 2	Day 3	Day 4	Days 5 -8	Days 9-10
DY	9	9	8	6	3	1

Note: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown based on the development of acidic conditions through fermentation with fatty acids as the daughter products; risk numbers are shown for each cell in the table and relates to a different forms of acidulolytic corrosion. This type of corrosion would be more lateral and generalized and would more likely lead to a micro-fracturing which would cause increases in porosity of the metal walls.

Acidulolytic corrosion (AC) would have a more generalised effect on the steel but would not necessarily cause perforations before there was a general structural collapse in the metal walls. This would be represented as a separate risk to the perforation risk.

13 HAB-BART testers to detect MIF

This tester may function for as long as 8 days with one of two possible reactions. Table HAB-1 lists the relative plugging risk (PR) using the time lapse (days) to the reaction signatures (UP, oxidative aerobic; DO, reductive anaerobic). MIF risk is generated from this table for the risk of biomass generation that could lead to either biofilms forming on the walls, in the porous or fractured media and reducing flow rates or plugging that could prevent flow completely. With this biomass generation there is also a much greater potential for the expanding biomass to cause radical changes to the water quality and also cause unacceptable dispersion of bacteria from the wells into the produced groundwater through collapsing biomass.

Table HAB-1, Risk Analysis Biofouling (MIF) based upon time lapse and reaction type

	Day 1	Day 2	Day 3	Day 4	Days 5	Days 6	Days 7-8
UP	9	9	8	5	3	1	1
DO	9	7	5	5	4	4	1

Note: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown based on the development of reduction in the tester from the bottom up (UP) in the case of aerobic bacteria dominating the sample or from the top down (DO) in the event of anaerobic bacteria dominating; UP reactions are commonly associated with oxidative conditions that commonly means the free presence of oxygen in the water; DO reactions are associated with reductive conditions; only one reaction can occur in the HAB-BART tester; plugging risk (PR). In common with the PIP and AC corrosion assessments the PR generates scales of risk from 1 to 9 with the most severe risk having the higher numbers.

14 IRB-BART testers to detect MIF

This tester may function for as long as 8 days with one of two possible reactions. Table IRB-1 lists the relative plugging risk (PR) using the time lapse (days) to the reaction signatures (CL, oxidative clouding; FO, reductive foam ring around BART ball; BR, oxidative brown slime ring around BART ball; BC, oxidative brown clouding; BG, redox front brown dense slime – gel; BL, reductive black liquid).

MIF risks associable to iron related bacteria (IRB) are more complex due to the greater variety of reaction pattern signatures that can be observed during testing. Essentially the reaction patterns give an indication of the environment within the water well that could generate biofouling. All of the other BART testers are selective in the types of reactions that can be observed but the IRB-BART has a greater diversity in the possible reactions that are seen. Examples of this is given below as the major types that can be observed: (1) CL generally relates to the establishment of oxidative conditions which along with TOC can create conditions where biomass can be generated very quickly but not necessarily with a significant ferric-iron content; (2) FO indicates that conditions at the sampled site were relatively reductive and, while the biomass may not grow so quickly, there is a greater potential for MIF events; (3) both the BC and BR reactions which commonly occur as secondary reactions indicate the conditions are relatively oxidative and rich in iron that indicates that a greater MIF risk exists; (4) BG reaction usually occur as secondary reactions that last from one to two days before moving on to a BC or a BL, when this reaction is observed then it should be considered that the sample came from a redox front that was rich in iron and TOC; (5) BL tends to be a terminal reaction that indicates that a broad spectrum of enteric and pseudomonad bacteria are active under organically rich moderately reductive conditions where significant growth in biomass can still occur.

With this biomass generation associated with ferric-rich concretious growths then there is also a much greater potential for the expanding biomass to cause radical changes to the

water quality and also lead to an unacceptable dispersion of bacteria from the wells into the produced groundwater through collapsing biomass.

Table IRB-1, Risk Analysis Biofouling (MIF) based upon time lapse and reaction type

	Day 1	Day 2	Day 3	Day 4	Days 5	Days 6	Days 7-8
CL	9	6	4	2	1	1	1
FO	9	8	5	3	3	3	2
BR or BC	9	6	5	4	4	3	1
BG	9	9	7	4	2	1	1
BL	9	9	8	6	4	3	2

Note: Day number refers to the number of days preceding the observation of the specific reaction being recognized; there can be more than one of the reactions observed during the test period with the most common sequences being: CL-BC-BR, FO-CL-BL, CL-BC-BL, CL-BG-BC-BL; the oxidation-reduction potential of the sample may be gauged from the first reaction with CL indicating oxidative and FO reductive conditions; Numbers in the cells for each reaction type recognized shows the general level of biofouling risk base upon the time lapse to the occurrence of the reaction

15 Determination of Risk Assessment Biofouling (RAB) Potential based upon the BART testing as defined.

Four BART tests are used for the determination of risk assessment for biofouling (RAB) in water well systems. Risks are assessed by the numbers generated during the BART testing that does give recognized and acceptable positive detections. Risk assessment numbers are generated from the four tables given above. Table SRB-1 indicates the risk of corrosive pitting and perforation that may be generated by the releases of hydrogen

sulfide from the biomass. If a BB reaction is observed then pitting is more probable while a BT reaction will occur in the event of more lateral forms of corrosion that may include cavitation of the metals. Table APB-1 detects when there has been a reductive fermentative event leading to the generation of (mainly fatty) acid products that will then drop the pH down into a more acidic range and also provide feedstock for the activities of SRB (ORP range, -10 to -150 mV) or methane producing bacteria (ORP range, -100 to -200 mV). If the acidic products are not degraded but collect within the biomass then acidic forms of corrosion are possible leading to losses in structural strength and gains in porosity in metal surfaces. Table HAB-1 assesses the risk of getting plugging through biomass generation under aerobic oxidative (UP reaction) and anaerobic reductive conditions (DO reaction). This part of the RAB specifically relates to the bacteria capable of generating biomass leading to plugging, nodules, encrustations and tubercle formation. These all have a primary impact on the transmissivity of groundwater through the injection or extraction wells. Table IRB-1 now assesses that potential impact of iron related bacteria. These bacteria tend to accumulate ferric forms of iron often as coatings on the biomass. While the IRB do become major components in the biomass along with HAB, the biggest difference is that the IRB generate relatively tight thick ferric-rich capping over the biomass that makes effective treatments more challenging.

Risk Assessment Biofouling (RAB) relates to a number of potential impacts that bacteria can have through the generation of biomass and development of corrosive process. Biomass (MIF) growth can fundamentally cause losses in flow due to some type of plugging which reduces the designed efficiency of the well system. Corrosion (MIC) events particularly target metal surfaces and, in particular, those with a high iron content. Here the corrosion may precipitate a pitting leading to perforation of the retaining metal, or the corrosion can gradually weaken the structures within the metals leading to collapse. Tables SRB-1 and APB-1 relate to MIC risk potentials while the tables HAB-1 and IRB-1 relate to MIF risk potentials. Protocols for assessing the RAB therefore are designed to generate two numbers, one relating to the MIC risk and the other relating to the MIF risk. These will be addressed separately below,

15.1 RAB (MIC) calculation

This calculation of MIC is based upon Tables SRB-1 and APB-1 in each of which only one number can be generated per table. For the SRB the risk number generated would be based on either the BT or BB reaction which ever observed first but not both. In the event that both BT and BB occur then the BB reaction is considered dominant for the purposes of this RAB (MIC) equation. For the APB only one number can be generated since there is only one single number that can be generated. Therefore the RAB (MIC) would a two digit number where the first number related to Table SRB-1 and the second number related to Table APB-1. The highest possible number would be 99 where both the SRB and the APB were very active with positive detections on the first day. The lowest possible number would be 00 where neither the SRB nor APB were detected. Numbers in the range represent some level of MIF risk. Possible interpretations are listed in Table MIC-1 below.

Table MIC-1, Examples of RAB (MIC) Assessments

RAB (MIC)	Interpretation
99	SRB and APB very active which means corrosion very likely with pitting and localised lateral localised structural failures.
90	SRB very active but no APB detected means that corrosion will likely involve pitting followed by perforations
09	No SRB activity detected but APB very active which means local sites within the biomass may generate acidic forms of corrosion
73	SRB activity detected (day 4, BB) indicating a probability of pitting with a low level of APB activity
26	Low level of SRB activity (day 9, BT) but APB were detected as moderately active (day 4, DY) which could mean lateral acid-generated corrosion was present under the biomass.

Note: in the RAB (MIC) number there are always two digits the first of which refers to Table SRB-1 and the second to Table APB-1; first digit therefore relates to various forms of pitting that could be occurring particularly on metal surfaces; second digit refers the risks from acidic forms of corrosion generated by APB; worse case corrosion would be a 99 in which both the SRB and APB are very active and aggressive; best case scenario would be a 00 in which no bacterial activity associated with the generation of hydrogen sulfide or acids were detected.

15.2 RAB (MIF) calculation

Microbiologically influenced fouling is calculated as a risk using data generated from Tables HAB-1 and IRB-1. Only the first positive detection for each of these tables is used in the calculation of the RAB (MIF) as a two digit number in which the first number reflects the biofouling risk of plugging associated with the HAB and the second number is the first generated number for the IRB. The highest possible number would be 99 where both the HAB and the IRB were very active with positive detections on the first day of testing. The lowest possible number would be 00 where neither the HAB nor APB was detected by the end of the testing. Numbers in the range represent some level of MIF risk. Possible interpretations are listed in Table MIF-1 below

15.3 RAB Full Interpretation

A full RAB including both the MIC and MIF assessment could be summarised as XX-YY where XX was the risk generated for the MIC and YY was the risk generated for the MIF. By nature of an example, 73-16 would mean that a significant risk from MIC would be presented by the SRB (7) but not by the APB (3). For the MIF the significant risk would be created by the IRB (6) mainly as a ferric rich dense biomass but without significant activity by the HAB (1). In some cases bacteria may not be detected for specific MIC or MIF events in which case these would be recorded as zero (absent, not active). If a RAB had a risk assessment of 00-70 then this would mean that only HAB were detected in a reductive (anaerobic) mode which would imply TOC were present.

This HAB community would be the major source of any MIF which would probably take the form of low density biomass that could cause plugging but would be relatively easy to treat. The other tests were negative (0) and this would mean there was no corrosion risk (SRB and APB absent) and no IRB (and hence no ferric rich dense biomass).

Table MIF-1, Examples of RAB (MIF) Assessments

RAB (MIF)	Interpretation
99	HAB and IRB very active which means plugging is very likely with losses in specific capacity and declines in water quality.
90	HAB very active but no IRB detected means that plugging will would likely involve a "soft" biomass without any iron accumulations
09	No HAB activity detected but IRB very active which means iron rich hardened dense biomass would form causing erratic declines in specific capacity
73	HAB very active and anaerobic (day 2, DO) with IRB less active in a reductive environment (day 4, FO). This would indicate that the plugging was from a more dispersed anaerobic biomass
16	Low level of HAB activity (days 6 - 8) but with IRB were detected as active (day 2, BR/BC) which could mean the biomass was ferric iron rich with relatively low organic carbon with the biomass forming possibly at the redox front.

References

1) 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

2) Alford, G., Mansuy, N. and Cullimore, D.R. (1989) The Utilization of the Blended Chemical Heat Treatment (BCHT^{TM}) Process to Restore Production Capacities to Biofouled Water Wells. Proc. of the Third Outdoor Action Conference on Aquifer Restoration, Ground Water Monitoring and Geophysical Methods. Association of Ground Water Scientists and Engineers. Orlando, Florida. May, p. 229 – 237.

3) Cullimore D.R, Johnston, L.A and G. Alford (2007) Evaluation of the effectiveness of selected rehabilitation processes applied to wells impacted by microbially influenced biofouling, proceedings of the American Water Works Conference, Toronto, Canada

4) Cullimore D.R. (1990) Microbes in Civil Engineering Environments: Biofilms and Biofouling In Microbiology in Civil Engineering (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16730 1, p. 3 - 12.

5) Cullimore, D. Roy (2008) Preliminary Comparison of the Applicability of the HAB-BART system and the Agar Spreadplate Methods for the Quantification of Bacterial Populations in Selected Dilutions of Pure Cultures of Three Bacterial species. Journal of Environmental Micropaleontology, Microbiology, and Meiobenthology Volume 3 31 to 43

6) Cullimore, D. Roy and Johnston, Lori (2007) "Rusticles on the *RMS Titanic"* JMBA published in Global Marine Environment May 2007, pages 10 to 12.

7) Cullimore, D.R, Pellegrino, C and Lori Johnston (2001) RMS Titanic and the Emergence of New Concepts on Consortial Nature of Microbial Events; Rev Environ Contam Toxicol 173:117 – 141

8) Cullimore, D.R. (1990) An Evaluation of the Risk of Microbial Clogging and Corrosion in Boreholes In Water Wells Monitoring, Maintenance, Rehabilitation (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16750 1, p. 25 - 34.

9) Cullimore, D.R. (1993) Practical Manual of Groundwater Microbiology. Lewis Publishing, Chelsea, Michigan ISBN 0-87371-295-1, pp. 403. (it has been designated as a best seller by the publishers)

10) Cullimore, D.R. (2000) Practical Atlas for Bacterial Identification published by Lewis Publishers / CRC Press, Boca Raton, Florida. (it has been designated as a best seller by the publishers)

11) Cullimore, D.R. (2005) Sulphur Reducing Bacteria chapter 4 in Anonymous (1995) Problem Organisms in Water: Identification and Treatment (organized by Organisms in Water Committee), AWWA Manual M7, published by American Water Works Association, Denver, Colorado. 153 pages

12) Cullimore, D.R. (2008) Practical Manual of Groundwater Microbiology. Lewis Publishing, Chelsea, Michigan, pp. 590.

13) Cullimore, D.R. and Legault, T. (1997) Microbiological Investigations of Water Wells in the Municipal District of Kneehill, Alberta. Rural Water Development Program, PFRA, Canada Agriculture, p. 27.

14) Cullimore, D.R. and Lori Johnston (2008) "Microbiology of Concretions, Sediments and Mechanisms Influencing the Preservation of Submerged Archaeological Submerged Archaeological Artefacts" Int J Histor. Archeol. In press

15) Cullimore, D.R. and Lori Johnston, (1999). The Fate of the Iron: More Lessons from the Titanic Tragedy. Maritime Reporter and Engineering News, August Edition, 20 & 66pp.

16) Cullimore, D.R. and Lori Johnston, (2000). Biodeterioration of the *RMS Titanic*. Canadian Chemical News/ L'Actualite Chimique Canadienne (ACCN), Ottawa, Ontario, 52 (10) 14 - 15

17) Cullimore, D.R. and Lori Johnston, (2000). Microbial Issues in Water Using an Innovative Detection System. Water Conditioning & Purification Magazine. November Issue, 71-78pp.

18) Cullimore, D.R. and Lori Johnston, (1999). The Fate of the Iron: More Lessons from the Titanic Tragedy. Maritime Reporter and Engineering News, August Edition, 20 & 66pp.

19) Cullimore, D.R. and Lori Johnston. (2000). The Impact of Bioconcretious Structures (Rusticles) on the *RMS Titanic*: Implications to Maritime Steel Structures. Proceedings of the 2000 Annual Meeting of the Society of Naval Architects and Marine Engineers, Vancouver, Canada, 9-1 to 9-16pp.

20) Cullimore, D.R. and Lori Johnston. (2000). The Impact of Bioconcretious Structures (Rusticles) on the *RMS Titanic*: Implications to Maritime Steel Structures. Proceedings of the 2000 Annual Meeting of the Society of Naval Architects and Marine Engineers, Vancouver, Canada, 9-1 to 9-16pp.

21) Cullimore, D.R., Abiola, A.T., Mnushkin, M. and Reihl, J. (1990) Evaluation of the Potential Biofouling of Water Wells, Newcastle, New Brunswick Regina Water Research Institute Report # 64c, p. 45.

22) Cullimore, D.R., Lebeden, J. and Legault, T. (1997) Investigation of Bacterially Driven Biofouling of Water Wells in Central Alberta, Canada. In Proceedings of the AGWSE National Education Program "Biological Aspects of Ground Water" Las Vegas, September. pp. 39 - 40.

23) Cullimore, D.R., Legault, T, Keevil, B., and Alford, G. (1997) The Laboratory Synthesis of Iron Related Bacterial Clogging in Well Screen and Gravel Pack In Proceedings of the AGWSE National Education Program "Biological Aspects of Ground Water" Las Vegas, September, p. 33 -34.

24) Cullimore, D.R., Mansuy, N. and Alford, G. (1990) Studies on the utilization of a Floating Intercedent Device (FID) in the Biological Activity Test (BATS) to Identify the Presence and Activity of Various Bacterial Groups in Groundwater. Proc. First Int. Symp. on Microbiology of the Deep Subsurface, USDOE, Orlando, Fl., p. 55.

25) Cullimore, D.R., Pellegrino, C., and Lori Johnston,(2001). *RMS Titanic* and the Emergence of New Concepts on Consortial Nature of Microbial Events. Rev. Environ Contam. Toxicol 173:117-142.

26) Cullimore, Roy (1999) Microbiology of Well Biofouling, published by Lewis Publishers / CRC Press, Boca Raton, Florida. 435 pages

27) Culllimore, D. R., and McCann, A. E. (1978) The Identification, Cultivation and Control of Iron Bacteria in Ground Water, Publ. In Aquatic Microbiology (ed., Skinner, F.A. and J. M. Shewan), Academic Press, New York, p. 219 - 261.

28) Garzke, W.H., Brown, D.K., Matthias, P.K., Cullimore, R., Wood, D. Livingstone, D, Leighley, H.P., Foecke, T., and A. Sandiford (1997) <u>*Titanic*</u>, The Anatomy of a Disaster, A Report from the Marine Forensic Panel (SD-7) Proceedings of the 1997 Annual Meeting of the Society of Naval Architects and Marine Engineers, Ottawa, Canada.

29) Gilman G. Violette Roy Cullimore Patrick Wall (2006) Successful rehabilitation of sand and gravel wells, Wilmot Park Well field, Fredericton Aquifer, Fredericton, New Brunswick. Proceedings of the CANADIAN Water Works Association (Atlantic Region), Halifax

30) Mansuy, N., Nuzman, C. and Cullimore, D.R. (1990) Well Problem Identification and its Importance in Well Rehabilitation In Water Wells Monitoring, Maintenance, Rehabilitation (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16750 1, pp. 87 - 99

31) Mansuy, Neil (1999) Water Well Rehabilitation, a Practical Guide to Understanding Well Problems and Solutions (Sustainable Well Series editor, D. Roy Cullimore) Lewis Publishers & CRC Press, Baton Rouge, FL 174pp

32) Rowe, K., Hrapovic, L., Kosaric, N., Cullimore, D.R. and Quigley, R.M. (1994) A Laboratory Investigation into the Degradation of Dichloromethane, Report conducted for the Interim waste Authority Ltd., Ontario by Geotechnical Research Center, University of Western Ontario, pp. 145.

33) Church, R., Warren, D., Cullimore, R., Johnston, L., Schroeder, W., Patterson, W., Shirley, T., Kilgour, M., Morris, N., and J. Moore (2008) A Study of Living History Deep WWII Shipwrecks in the Gulf of Mexico, PAST Foundation Publications, Columbus, OH. Pp239

B Technical Support Documents

a) About the BART testers, general information DBIMANG06 December 20, 2006

b) APB-BART Protocol DBASOPO6 April 2, 2006

c) BART commentary from various users, June 7, 2006

d) BART-SOFT version 6.08 released June 2008

e) Comparison of BART™ Testers with Other Microbiological Tests August 20, 2005

f) Concept behind BART testers October 17, 2005

g) Confirmation of the Selective Media Composition for BART testers March 18, 2008

h) DN-BART Protocol DBDNSOP06 April 2, 2006

i) HAB-BART Protocol DBHSOP06 May 4, 2006

j) HAB-BART System™ FOR THE DETECTION OF HETEROTROPHIC AEROBIC

k) BACTERIA (HAB) Using the HAB-BART Tester™ and HAB-BART Reader™ Environmental Technology Verification document August 4, 2004 pages 23

l) IRB-BART Protocol DBISOP051 May 3 2005

m) IRB-BART™ TESTER FOR THE DETECTION AND EVALUATION OF IRON BACTERIA IN WATER Environmental Technology Verification document July 27, 2002 pages 45

n) Relationship of time lapse to bacterial populations created by daily observation of selected BART testers October 17, 2006

o) SLYM-BART Protocol DBLSOP05 March 9, 2005

p) SRB-BART Protocol DBSSOP06 May 5, 2006

q) SRB-BART™ TESTER FOR THE DETECTION AND EVALUATION OF SULFATE REDUCING BACTERIA IN WATER Environmental Technology Verification document July 27, 2002 pages 53