



Report Date: 8/30/2022

Microbiological Analyses



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Company Information

Bond Water Technologies, Inc.
630 E. Diamond Avenue; Suite J/K
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Job Site:

VA-011
1824 Industry Dr.
Culpepper, VA

Batch Number: 220823021

Sampled By: Marshall
P.O. Number:
Report Status: Original

Client Sample ID: VA-011

Lab Sample ID: 338678

Collection Date: 8/22/2022

Receive Date: 8/23/2022

Location: CT

Analyte	Test Code	Media Type	Sample Result	Units	Detection Limit	Analysis Date	Priority
Legionella pneumophila SG1	L001 - Legionella Non-Potable Water	GVPC	None Detected	CFU/mL	10	CFU/mL	8/30/2022
Legionella pneumophila SG2-14	L001 - Legionella Non-Potable Water	GVPC	None Detected	CFU/mL	10	CFU/mL	8/30/2022
Legionella non-pneumophila	L001 - Legionella Non-Potable Water	GVPC	100	CFU/mL	10	CFU/mL	8/30/2022

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Report Notes Applicable to All Analyses

- Sample transit delays occurring between the time of collection and receipt at the laboratory can affect the validity of test results. EST's recommendations for the collection, transport, and shipping of microbiological samples are located at www.estechlab.com. See the document titled "Sample Collection Guidelines" for analysis-specified recommendations. EST does not recommend transit exceeding referenced guidelines. In the event of a sample transit delay, designated account contacts are notified, and samples are analyzed only upon documented processing requests.
- Data are not corrected based on results for blank samples. Results relate only to items tested. Results apply to samples as received.
- Raw counts (available upon request) are used to calculate test results using all significant figures. Results found at or above the analytical sensitivity are reported to three significant figures; amounts below the analytical sensitivity are listed as None Detected.
- Analytical Sensitivity is defined as the lowest concentration that can be detected by a test method based on the amount or portion of sample analyzed and is reported without rounding. For qualitative samples, results found at or above this level are reported as "Present" and amounts below this limit are reported as "Absent".
- Samples collected by EST's Industrial Hygiene Department are indicated on reports by the suffix, /EST, appearing after the "Sampled By" field. EST is responsible for all the information provided on issued reports unless information is provided by the customer. Chain of Custody (COC) records accompanying samples submitted to laboratory are scanned and included with issued reports; see COC records for traceability of provided information including but not limited to sample collection time, sample rate, transport conditions, sampling media, and lot numbers. If pertinent information needed for sample processing or calculation of reported results is omitted from a COC record, customers are contacted for verification and information is recorded on the submitted COC record or a proxy COC record if one has not been provided.

Abbreviations

- ANAB = ANSI National Accreditation Board; accreditation fulfills the requirements of ISO/IEC 17025:2017.
- Media Types: BART = Biological Activity Reaction Tester, BCSA = *Burkholderia cepacia* Selective Agar, BCYE = *Legionella* Buffered Charcoal Yeast Extract Agar, Cetrimide = *Pseudomonas aeruginosa* Selective Agar, GPVC = *Legionella* BCYE Selective Agar with antibiotics (Glycine, Vancomycin, Polymyxin, and Cycloheximide), Chromogenic Media = chromogenic differential media for presumptive pathogen detection (*E. coli*, *Klebsiella/Enterobacter/Serratia* spp., *Enterococcus* spp., *Proteus/Morganella/Providencia* spp., *Staphylococcus aureus*, *Pseudomonas* spp., *Staphylococcus saprophyticus*, *Candida* spp., *Citrobacter* spp.), Leeds Medium = *Acinetobacter* Selective Medium, ME* = Malt Extract fungal Identification Agar with 0.01% Chloramphenicol, MHA = Mueller Hinton Agar, Microfilm EBEC = Enterobacteriaceae/*E. coli* Selective Media, Microfilm TCEc = Total Coliform/*E. coli* Selective Media, Microfilm YMC = Yeast and Mold Count Media, , R2A = Reasoner's 2A Bacterial Agar, SMA = Standard Methods Agar (a.k.a. Plate Count Agar), SSA = *Stenotrophomonas* Selective Agar, TSA = Tryptic Soy Agar, TSA* = Tryptic Soy Agar with 0.005% Cycloheximide, TSLT = Tryptic Soy Agar Contact Plate with Lecithin and Tween 80, Selective 7H11 = Middlebrook 7H11 Selective Agar for *Mycobacterium* species.
- Miscellaneous: C = Celsius, CFU = Colony Forming Unit, F = Fahrenheit, g = gram, L = liter, m = meter, ml = milliliter, SG = serogroup, HPC = Heterotrophic Plate Count, NTM = Nontuberculous mycobacteria, N/A = Not Applicable, N/R = Not Requested.

Bacteria – Standard Heterotrophic Plate Counts for Water Samples

- Test Code B010: Aerobic, heterotrophic plate bacteria counts using Microfilm APC standard methods agar (AOAC Method 051702).
- Test Code B100: Aerobic, heterotrophic plate counts are obtained using SMA plates (Standard Methods 9215C).
- Test Code B011: Anaerobic, heterotrophic plate counts are obtained using SMA agar plates (Standard Methods 9215C) under anaerobic gas generating systems.

Bacteria – Plate Counts for Air, Swab, Bulk-Solid, and Contact Plates

- Test Codes: B002 (Air), B004 (Swab), B007 (Bulk-solid), B027 (Contact)
- Plate counts are routinely obtained utilizing SMA agar plates for air, swab, and bulk-solid samples as described above.
- Other agars may be utilized upon customer request (e.g., TSA plates incubated for 3-5 days at 35°C or R2A plates incubated for 5-7 days at 30°C).
- Bacteria plate counts are obtained for bulk-liquid and bulk-solid samples using the spread plate method.
- Surface contact plates (e.g. TSLT) are incubated directly at room temperature for 3-5 days unless otherwise requested and noted.

Legionella Culture Analysis

- Test Codes: Non-potable L001 (Bulk-Liquid/Water), L002 (Swab), L003 (Bulk-Solid), L099 (Air)
- Test Codes: Potable L011, LCMS (Bulk-Liquid/Water), L012 (Swab), L013 (Bulk-Solid)
- Total Viable *Legionella*: Viable *Legionella* counts (*Legionella pneumophila* SG1, *Legionella pneumophila* SG2-14, or *Legionella* non-*pneumophila* species) are obtained using an in-house modified method based on CDC and ISO 11731:2017(E) procedures for the recovery of *Legionella* from the environment. *Legionella* non-*pneumophila* species include *L. anisa*, *L. bozemanii* 1 & 2, *L. dumoffi*, *L. feelei*, *L. gormanii*, *L. jordanis*, *L. longbeachae* 1 & 2, and *L. micdadei*.

Environmental Pathogen Monitoring - Listeria and Salmonella Immunoassay Tests

- Test Code B041: *Listeria*. Swabs are enriched in PDX-LIB™ indicator broth for enhanced recovery and selection.
- Test Code B042: *Salmonella*. Swabs are enriched in PDX-SIB™ indicator broth for enhanced recovery and selection.
- Test methods AOAC Research Institute approved for monitoring environmental surfaces.
- Presumptive positive cultures are confirmed using pathogen specific selective agars.

Fungi

- Test Codes Count & Identification: F003 (Swab), F005 (Bulk-Solid), F007 (Bulk-Liquid), F014 (Contact Plate)
- Test Codes Count Only: F004 (Swab), F006 (Bulk-Solid), F008 (Bulk-Liquid), F013 (Contact Plate)
- Fungal samples processed for counts and identification are obtained directly from listed processing agars.
- Fungal counts only (no identification) are obtained using Fungal Count Yeast and Mold Count (YMC) Microfilm™
- All samples are incubated aerobically at 29°C for 7 days unless otherwise requested.

Pathogen Screen Culture Analysis – Potable Water Samples

- Test Code CMS1: Waterborne pathogen screen.
- Test Code LCMS: Waterborne pathogen screen accompanying *Legionella* analysis on BCYE and GVPC agar.
- Analyses on pathogen specific selective agars for the following organisms: *Acinetobacter* species, *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, Rapidly-growing non-tuberculosis mycobacteria, Fungal Count (mold and yeast) and Mold Identification (Genus-level; common *Aspergillus* species).
- Incubations at optimal growth temperatures for each pathogen and presumptive positive cultures are confirmed utilizing pathogenic specific biochemical tests.

Non-tuberculous Mycobacteria (NTM) Rapid-growing Mycobacteria (RGM) Screen – Culture Method

- Test Code: CMS1, LCMS, B043 – Culture method
- Detection of rapid-growing (\leq 7-day) non-tuberculous mycobacteria on 7H11 Selective agar with acid-fast stain confirmation.

Non-tuberculous Mycobacteria (NTM) vPCR Analysis

- Test Code: P002 - Viable PCR*
- Detection of *Mycobacteria* in environmental water samples. This rapid vPCR screen provides a total count for all non-tuberculous mycobacteria species and for each of the two most clinically relevant slow-growing mycobacteria (SGM) species, *avium* and *intracellulare*, which would require a four-week incubation by the culture method.
- Results obtained using an in-house modified method by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).
- Analysis by vPCR includes viable but non-culturable (VBNC) *Mycobacteria* not detected by routine viable culture analysis. In addition,
- VBNC *Mycobacteria* may be present in a damaged state in response to disinfectants or other toxic conditions, but they may be unable to recover and grow on the surface of an agar petri dish (i.e., non-culturable), and appear as a none detected viable *Mycobacteria* culture result.
- If damaged *Mycobacteria* are introduced to a more favorable environment, such as an amoeba living in an environmental water source or into a human lung macrophage, they may be able to recover and even proliferate. In humans, following inhalation of *Mycobacteria* from a contaminated source, recovery and growth in lung tissue may result in a pulmonary disease infection.
- Analysis by vPCR allows the simultaneous detection of both culturable and VNBC *Mycobacteria* from environmental samples to ensure an accurate risk assessment.
- vPCR NTM analyses is pending ANAB scope of accreditation for test methods.

Legionella vPCR

- Test Code: P001 - Viable PCR
- Detection of non-viable discrimination of *Legionella pneumophila* SG1-15 and *Legionella pneumophila* SG1 from environmental water samples.
- Results obtained using an in-house modified method based on ISO 12869:2019; Water quality – Detection and quantification of *Legionella* spp. and/or *Legionella pneumophila* by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).
- *Legionella* detected by vPCR include those that are viable but non-culturable (VBNC) bacteria not detected by routine viable culture analysis.
- VBNC *Legionella* may be present in a damaged (but not dead) state in response to disinfectants or other toxic conditions, where the bacteria were unable to recover and grow on the surface of an agar petri dish (i.e., non-culturable), and appear as a none detected viable *Legionella* result.
- If damaged *Legionella* are introduced to a more favorable environment, either an amoeba in an environmental water source or into a human lung macrophage by inhalation, they may be able to recover and even proliferate. In humans, recovery in lung tissue may result in a Legionnaire's disease infection.
- vPCR allows the simultaneous detection of both culturable and non-culturable *Legionella* from environmental samples to ensure a more accurate risk assessment.

Microbial Corrosion Screen (Water, Swab, or Solid/Sludge)

- Biological Activity Reaction Test (BART) biodetectors, manufactured by Dryocon Bioconcepts Inc. (DBI), monitor biological activity of specific groups of bacteria as listed in the table below. Organism specific biodetectors are inoculated with sample and are monitored for reaction changes described by manufacturer's certificates of analysis (COA) for each lot until activity is positive or determined to be not aggressive. Upon observation of a positive reaction, a semi-quantitative approximation (CFU/ml) of the microbial population being tested is matched and aggressivity is assigned as either aggressive, moderate, or not aggressive.

BART™ Biodetector	BART™ Microbial Population	Test Codes (Water/Swab/Bulk)	Aggressive	Moderate	Not Aggressive
APB	Acid Producing Bacteria	M001, M011, M021	475,000 - 14,000	4,500 - 75	10 - <2
IRB	Iron Related Bacteria	M003, M013, M033	570,000 - 9,000	2,200 - 25	8 - <1
SLYM	Slime Forming Bacteria	M004, M014, M024	1,750,000 - 67,000	13,000 - 500	100 - <20
SRB	Sulfate Reducing Bacteria	M005, M015, M025	2,200,2000 - 6,000	1,400 - 75	20 - <1
FLOR	Fluorescent Pseudomonads	M006, M016, M026	2,000,000 - 4,000	800 - 35	7 - <1
ALGE2	Algae	M008, M018, M028	500,000 - 10,000	1,000 - 100	<100
BART™ Test	BART™ Microbial Population	Test Codes (Water/Swab/Bulk)	Aggressive	Not Aggressive	
DN2	Denitrifying Bacteria	M002, M012, M022	>100,000 - 1,001	1,000 - <1	
N2	Nitrifying Bacteria	M007, M017, M027	>100,000 - 1,001	1,000 - <1	

*DBI BART™ screens are not covered under ANAB scope of accreditation for test methods.

Endotoxin (Water)

- Test Codes E010 Endotoxin Limit 10 EU/ml, E020 Endotoxin Limit 20 EU/ml.
- Test result less than test limit = Endotoxin Absent. Test result greater than or equal to test limit = Endotoxin Present.
- Assay performed using gel clot limulus amebocyte lysate (LAL) test for bacterial endotoxin.

Microbiological Analyses Endnotes Revision 1-5