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08 April 2009

Yone Akagi
Portland Water Bureau
1900 N. Interstate Ave
Portland, OR 97227

Subject: LETTER OF TRANSMITTAL
Portland Water Bureau LT2 Variance Study Plan

Dear Yone,

Enclosed please find Analytical Services, Inc.'s (ASI) proposed LT2 Variance Study Plan for submission to the USEPA. Of course, the plan is subject to Portland Water Bureau's (PWB) review and comment, and ASI is willing to discuss any and all aspects of the Plan.

If you have any questions, please contact Colin Fricker, Ph.D. or me at any time.

Sincerely,

ANALYTICAL SERVICES, INC. (ASI)

Paul S. Warden
Vice President & Director of Operations

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Portland (OR) Water Bureau LT2 Variance Study Plan

Introduction

The City of Portland, Oregon, Water Bureau (PWB) is an unfiltered Community Water System serving over 500,000 customers, which meets the filtration avoidance criteria in the Surface Water Treatment Rule. As a Long Term 2 Enhanced Surface Water Treatment Rule (LT2) Schedule 1 Public Water System (PWS), PWB has completed its required 24 months of raw water *Cryptosporidium* monitoring under LT2[(1)]. However, the PWB does not wish to be classified as requiring at least 2-log *Cryptosporidium* inactivation and accordingly will pursue a variance from the requirements of the Rule.

The LT2 Rule states that "If an unfiltered PWS could show a raw water *Cryptosporidium* concentration level 3-log lower than the Bin 1 cutoff for filtered PWSs (i.e., below 0.075 oocysts per 1000L), this could demonstrate that that no treatment for *Cryptosporidium* is necessary"[(1)]. The City of Portland intends to demonstrate that no treatment for *Cryptosporidium* is necessary for water from its Bull Run watershed. PWB will intensively sample its source water to demonstrate that it contains less than 0.000075 oocysts per Liter, which is 3-log lower than the Bin 1 cutoff (<0.075 oocysts/L) for filtered public water systems. The City also intends to apply continuous historical and concurrent monthly 50 liter *Cryptosporidium* monitoring data towards the variance.

Project Team

PWB has assembled a LT2 variance study team, which is directed by Ms.Yone Akagi, Regulatory Compliance Manager. Mr. Ryon Edwards, Environmental Technician II, will perform sample collection and coordinate day to day operational details, supported by Ann Richter, Environmental Technician II. Ms. Akagi is the project manager, however, Mr. Edwards will serve as the primary contact person for this study. The PWB project team, with the support of PWB management and their stakeholders, has detailed its requirements for the LT2 variance process, issued a Request For Proposal, and through this process, selected Analytical Services, Inc. (ASI) of Williston, Vermont to perform the necessary consulting and microbiological testing. ASI is nationally regarded as an expert laboratory regarding *Cryptosporidium* and *Giardia*. ASI was one of the first laboratories audited and approved by the USEPA under the LT2 Laboratory Quality Assurance Program for *Cryptosporidium* testing, and in 2008 we were reaudited and reapproved by the EPA. ASI was approved for *Cryptosporidium* and *Giardia* testing during the Information Collection Rule (ICR) and was selected to participate in the ICR Supplemental Survey. ASI has provided LT2-compliant *Cryptosporidium* data since 2001. ASI is also accredited under the National Environmental Laboratory Accreditation Program (NELAP) by the Florida Dept. of Health to perform Method 1623 (and other parameters) and is also certified by the Dept. of Health in Vermont, Connecticut, and New Mexico.

ASI's Project Team will be headed by Dr. Colin Fricker, ASI Technical Director. Dr. Fricker is an internationally recognized expert in the microbiology of water and public water

supplies, with particular regard to *Cryptosporidium*, and has over 30 years experience in environmental microbiology in public and private sectors. He has worked with ASI since 2001. Mr. Paul Warden will serve as ASI's Project Manager for the City of Portland project, and will be ASI's primary contact person. Mr. Warden has over 19 years experience in commercial laboratories and as Vice President and Director of Operations, he is responsible for daily operations at ASI and directs ASI's Client Services department. Mrs. Courtney Audy, ASI Quality Assurance Officer, will serve as Project Quality Assurance Manager. Ms. Carolyn Brault, ASI Staff Microbiologist, coordinates daily laboratory work and schedules for the protozoa analysis section of ASI. Ms. Brault is EPA Approved as a Principal Analyst for LT2 and began working at ASI in 2004.

Preparatory Work

In January 2009, Dr. Fricker completed a site visit to PWB, including inspection of the sample collection point in the Bull Run watershed. Following this visit an investigation was begun to determine the most appropriate procedures for sample collection and analysis. Assessment of the Pall Gelman Envirochek HV filter and the IDEXX Filta-Max *xpress* filters was conducted, focusing on high volume throughput and *Cryptosporidium* recovery. The pleated configuration of the polyester, hydrophilic, track etched membrane of the HV filter has 1300 cm^2 effective filtration area[(2)]. The HV filter allowed greater throughput with less back pressure due to partial filter clogging than did Filta-Max filter modules, which are of a compressed foam depth filter configuration. Alternate elution regimes were also investigated, including the use of warm elution solution as per the U.K. method[(3)] and the use of a pre-elution step with sodium hexametaphosphate prior to the standard elution procedure. Polyphosphates are negatively charged and have been reported to reduce the zeta potential of particles suspended in water[(4)]. Sodium hexametaphosphate may help minimize the adhesion of microbes to filter surfaces and several researchers have examined the benefit of incorporating sodium hexametaphosphate in sample preparation steps[(5,6,7,8)].

After a review of the data generated, it was decided to perform a Tier 1 validation study to validate the performance of the method as modified for this study. Under EPA's Performance Based Measure System (PBMS), Method 1623 is a performance based method and therefore allows method modifications provided the laboratory can meet applicable quality control criteria as specified in Section 9.1.2 and Section 21, Tables 2 – 4 of Method 1623[(9)]. It was determined that the modifications to be included in the Tier 1 study were (a) using Pall Gelman HV filters to filter 200L, (b) pre-elution of the filters with 5% sodium hexametaphosphate solution and (c) filtration flow rate increased to four liters per minute (4 LPM).

Tier 1 Validation Study - For this study, five (5) reagent water samples were collected from ASI's laboratory deionized water system. All five (5) samples were collected as 190L filtered at a pressurized tap, followed by 10L collected in bulk. During sample collection, flow rate was controlled using a flow control valve to ensure it did not exceed 4 LPM and the volume sampled was measured using a calibrated flow totalizer.

In addition to the required reagent water samples, the field sample (FS), matrix spike (MS) and matrix spike duplicate (MSD) samples were also collected and analyzed.

These samples were collected at PWB's Bull Run sampling site, the same sampling location as used for LT2 monitoring and that will be used for the LT2 variance study. The FS/MS/MSD were collected by PWB staff using the standard approach for Method 1623 samples greater than 10L; the FS was field filtered to 200L and the MS/MSD were field filtered to 190L each, with a 10L grab sample collected for each. During sample collection, flow rate was controlled by a valve and total filtrate volume was recorded.

The volume of each bulk sample was confirmed by weight on ASI's calibrated laboratory scale and adjusted to 10L (±0.1L). Four of the 10L reagent water grab samples and the MS/MSD samples were spiked with EasySeed (BioTechnology Frontiers, AU). The method blank 10L bulk sample was not spiked, but was otherwise processed as per the spiking procedure to show the absence of contamination. The field sample was processed identically to the other samples, with the exception of the spiking procedure. All seven bulk samples (the 10L portions) were filtered through the respective HV filters in accordance with ASI's standard operating procedure for matrix spike samples. Briefly, this includes thorough mixing using a disinfected stir bar and magnetic stir plate, filtering the sample under vacuum pressure through the HV filter, followed by a one liter reagent water rinse of the cubitainers, with the rinsate also filtered. During the filtration of the spiked reagent water samples, the flow rate through the HV filter was precisely controlled at 4 LPM using a calibrated flow rotameter with an adjustable flow control valve. The same equipment and procedure was used for the MS/MSD samples although the flow rate was lower due to the particulate load on the filter, as is commonly seen in MS samples.

Following spiking, all filters were pre-eluted with a 5% (weight to volume) solution of sodium hexametaphosphate for five (5) minutes at 600 oscillations per minute. The sodium hexametaphosphate was pulled through the filter via the outlet port by vacuum pump and the filter was rinsed with DI water, also evacuated by vacuum. Following this pre-elution step, samples were processed in accordance with EPA Method 1623. The results of the Tier 1 study are shown in Table 1 and a comparison of the Tier 1 sample results to the EPA Method 1623 acceptance criteria is shown in Table 2.

Table 1. Results of PWB Tier 1 study using HV filters, 200L samples, 4 LPM and sodium hexametaphosphate pre-elution. (*Cryptosporidium* percent recovery data are highlighted in blue).

		Total Count		**Spike Dose**		**Percent Recovery**	
ASI #	IPR#	*Giardia*	*Crypto*	*Giardia*	*Crypto*	*Giardia*	*Crypto*
35013-01	Reagent Spike 1	60	51	101	99	59.4	51.5
35013-02	Reagent Spike 2	59	51	101	99	58.4	51.5
35013-03	Reagent Spike 3	68	47	101	99	67.3	47.5
35013-04	Reagent Spike 4	62	60	101	99	61.4	60.6
35013-05	Blank (5)	0	0	N/A	N/A	N/A	N/A
35026-02	PWB-MS	63	38	100	99	62.0	38.4
35026-03	PWB-MSD	50	32	100	99	49.0	32.3
35026-01	PWB-FS	1	0	N/A	N/A	N/A	N/A

Table 2. Comparison of Tier 1 results and EPA Method 1623 acceptance criteria. (*Cryptosporidium* data are highlighted in blue).

	Initial Precision and Recovery				Matrix Spike / Matrix Spike Duplicate			
Protozoa	ASI Mean %R	1623 Accept. Range	ASI RSD	1623 Max. RSD	ASI Mean MS/MSD	1623 Accept. Range	ASI RPD	1623 Max. RPD
Giardia	61.6	24 - 100	6.5	49	55.5	15 - 118	23.4	30
Crypto.	52.8	24 - 100	10.5	55	35.4	13 - 111	17.1	61
RSD = Relative Standard Deviation = (SD / Mean) *100								
RPD = Relative Percent Difference = (Difference / Mean) *100								

The data generated in the Tier 1 study meet all acceptance criteria set forth in Method 1623.

For completeness, the Tier 1 data were also compared to historical data provided by PWB from their ongoing monthly *Cryptosporidium* monitoring (50 L samples), including matrix spike recoveries and laboratory Ongoing Precision and Recovery (OPR) results, from Clancy Environmental Consultants (St. Albans, VT). This comparison is presented below in Table 3.

Table 3. Comparison of the percent recovery of *Cryptosporidium* (%C) in historical 50L PWB monitoring data from Clancy Environmental Consultants (CEC) and 200L Tier 1 study conducted by Analytical Services, Inc. (ASI). (Comparable means are highlighted in same color).

Volume = 50L (CEC)		Volume = 200L (ASI)	
Matrix Spike (%C) (n=5)	Associated OPR (%C) (n = 5)	Matrix Spike (%C) (n = 2)	IPR (%C) (n = 4)
20.2	45.5	38.4	51.5
56.6	34.4	32.3	51.5
21.1	30.3		47.5
2.0	29.3		60.6
41.0	54		
Avg. = 28.1%	Avg. = 38.7%	Avg. = 35.3%	Avg. = 52.8%

Recovery of *Cryptosporidium* from matrix spike samples varies with sample water quality (in addition to laboratory and analyst-specific variables). The matrix spike results from the Tier 1 study, using 200L, 4 LPM and sodium hexametaphosphate, have a higher mean recovery and lower variability than do the matrix spike results from previous monitoring using the standard procedure (Table 3, above). In reagent water, the lowest *Cryptosporidium* recovery observed during the Tier 1 study exceeded all but one of the OPR recoveries that were associated with a Matrix Spike during routine monitoring Table 3, above), resulting in a substantially higher mean recovery.

In conclusion, despite the increased volume of the Tier 1 samples, the *Cryptosporidium* recovery and reproducibility data generated using the modified method compare favorably with the historical data generated for PWB. The Tier 1 study data, generated using modified method proposed for the PWB LT2 Variance Monitoring, meet all 1623 Method Modification requirements.

PWB intends to conduct its LT2 Variance Monitoring Program using up to 200L samples collected using Pall Gelman HV filters, which will be analyzed by ASI using the method modification described herein.

Sample Collection Plan

PWB intends to monitor its Bull Run watershed intake point by intensive sampling to demonstrate a *Cryptosporidium* concentration of less than 0.000075 oocysts per Liter. To accomplish this, PWB will collect regularly scheduled 200L samples over a one year period, resulting in approximately 54,000 Liters collected and analyzed. As per LT2 requirements, samples will be collected prior to any treatment. The sampling flow rate will be less than or equal to 4 LPM.

Samples will be collected on a predetermined schedule, as described below, to avoid any data bias. During the first, third and fourth weeks of each month during the monitoring period, five (5) 200L samples will be collected (three on Mondays and two on Tuesdays). The Monday samples will be returned to PWB and stored overnight, in a dedicated laboratory refrigerator at <10°C. The temperature of the refrigerator is monitored daily and recorded. The Tuesday samples will be collected and returned to PWB, where they will be shipped by overnight delivery to ASI. The second week of each month, six samples will be collected (three on Monday and three on Tuesday) using the same storage procedure described above. Once a month, a 50L sample will be collected and sent to ASI using the same collection and storage procedure as described above. This schedule will result in collection of approximately 54,000 L over the 12 month period, while providing cost efficiency (with respect to shipping) and allowing for resampling if required. A four day sampling window, as described in LT2, will be used for this project, to allow for severe weather events, illness, etc. Resampling, if necessary, will be performed within 21 days of notification, in accordance with LT2 guidelines.

All samples will be shipped by overnight courier in high quality coolers, typically consisting of a Styrofoam cooler (1.5 inch thick wall) inside cardboard outer box. For further protection, each filter will be inserted into a thermally insulated envelope prior to being put into the cooler. Re-freezable ice packs will be provided by ASI, commensurate for the season. ASI has tested this shipping configuration under extreme conditions and found it to be both practical and robust. Matrix spikes will be collected at a frequency of no less than one sample in 20, using the procedure documented above for the MS/MSD samples in the Tier 1 study (190L field filtered and 10L collected and shipped as a bulk sample).

A reserve stock of HV filters will be maintained at PWB to allow immediate resampling as necessary. Other supplies will be shipped by ASI to PWB on a regular schedule, with

sampling supplies to arrive at two weeks prior to sample collection to allow time for adjustment as necessary.

Laboratory Procedures

At ASI, each sample will be received by Sample Management and processed in accordance with ASI standard procedure, prior to login to ASI's Laboratory Information Management System (LIMS). Sample receipt processing includes measuring and recording sample temperature (using temperature blanks) and inspecting the sample for any nonconformances (temperature, presence or evidence of ice, hold time, volume, incomplete or missing paperwork, etc.). Any nonconformances noted will be recorded using ASI's Corrective Action Record system, which is summarized weekly by ASI's QA department for management review of trends. Any nonconformances noted will be communicated to PWB on the day of sample receipt to facilitate resampling plans as needed. The standard sampling week rotation is depicted in the table below.

Table. 4. Standard weekly rotation; monitoring and communication.

Monday	Tuesday	Wednesday	Thursday	Friday
PWB: Collect 3-200L samples. Store in dedicated lab refrigerator at PWB	PWB: Collect 2-200L samples. Ship 5 samples to ASI.	ASI: Confirm sample receipt and condition; advise if replacement samples required.	PWB: Collect replacement samples as needed. Ship to ASI.	ASI: confirm sample receipt and condition, if appropriate.

Analytical Method for PWB Samples - All ASI staff participating in this project will receive specific training for their responsibilities. All samples will be processed at ASI in accordance with the project specific protocol described above. The primary analytical modification that will be employed is the pre-elution with sodium hexametaphosphate described above. Two minor modifications, which are incorporated into ASI's standard procedure for Method 1623, will also be included on all samples. These are (1) adding 0.001% Tween 20 to the IMS microcentrifuge rinse step to prevent the formation of bubbles due to the surface tension, and (2) omitting the methanol fixation step, prior to staining the sample fluorescently labeled monoclonal antibodies in preparation for examination by fluorescence microscopy. Use of methanol is described by the manufacturer of EasyStain (BioTechnology Frontiers) as optional and we have found it can contribute to degradation (wrinkling) of (oo)cysts and have a slight detrimental effect on fluorescein isothiocyanate (FITC) staining.

For quality control during this study, every week that high volume PWB samples are received, ASI will perform an OPR using the "PWB sample protocol" described above, which includes Pall Gelman HV filters, 200L volume and sodium hexametaphosphate pre-elution. This commitment goes beyond the minimum recommendation to do high volume OPRs at a frequency commensurate the percent of high volume samples analyzed.

Sample results will be reported by ASI to PWB within 10 business days of sample receipt. The final report format is to be determined. A final report summarizing the study plan, protocols, and all analytical results will be submitted to the EPA by PWB upon completion of the monitoring.

Problematic Samples

In the event that a HV filter clogs prior to collection of 200L, a second filter will be installed and used to collect the remaining volume up to 200L. If the second filter clogs prior to completion of 200L, sampling will be considered complete as per LT2 requirements and no additional filters will be used for that sample.

If, during elution at ASI, a sample yields a packed pellet volume in excess of 0.5 mL, the sample concentrate will be divided into appropriate aliquots and resulting sub-samples processed through immunomagnetic separation, staining and microscopic examination. It is ASI's intent to analyze the entire volume of all samples submitted by PWB.

In the event that the Bull Run watershed is shutdown and not serving drinking water, no samples will be collected. The PWB project team and ASI will recommend the most appropriate schedule to make up the sample volumes.

Other unforeseen problematic situations that may arise will be fully documented and communicated promptly to the EPA, with the project team's recommended corrective action.

Summary

PWB has expended considerable resources to protect and monitor its high quality water source, the Bull Run watershed, and to maintain filtration avoidance status. PWB believes that a variance to LT2 treatment requirements is in the best interest of its customers and accordingly has contracted ASI to assist in the development and execution of a scientifically sound and defensible plan to generate the monitoring data required to pursue a LT2 variance.

PWB anticipates initiating monitoring the week beginning 20 April 2009, in anticipation of EPA approval of this plan. PWB respectfully requests prompt review and comment on this plan.

References

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