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USEPA Method 1623 and 1623.1

ASI Technical Document #110

Introduction

Cryptosporidium spp. and *Giardia intestinalis* are well-known waterborne pathogens that have caused disease outbreaks around the globe. The 1993 outbreak of cryptosporidiosis in Milwaukee and subsequent outbreaks have stimulated research regarding the occurrence and behavior of these pathogens in natural waters.[1] Substantial research efforts have been expended to identify factors related to pathogen levels in water supplies and to identify problematic aspects of traditional water treatment and handling.[1,2,3] Another research focus has been the development of improved sample collection methods and laboratory techniques for the recovery and detection of *Cryptosporidium* and *Giardia.*[4]

Method 1623 - General

This technical document summarizes the development of USEPA Method 1623 and presents options for the simultaneous detection of *Cryptosporidium* and *Giardia* (Method 1622 detects only *Cryptosporidium*).

Methods 1622/3 were developed to address some of the limitations of the Information Collection Rule (ICR) Protozoa Method that have been identified by various researchers.[2,5,6] For improved detection and enumeration of *Cryptosporidium* and *Giardia*, Method 1623 incorporates filtration, immunomagnetic separation (IMS) and immunofluorescence assay (FA) microscopy.[6,7] Organisms are confirmed using 4,6-diamidino-2-phenylindole (DAPI) vital dye staining and differential interference contrast (DIC) microscopy.

Analytical Services, Inc. (ASI) participated in the EPA's inter-laboratory method validation studies for Method 1622 (*Cryptosporidium*) in 1998 and Method 1623 (*Cryptosporidium* and *Giardia*) in 1999. ASI was also 1 of 6 laboratories nationally to participate in the EPA's ICR Supplemental Survey (ICRSS). ASI is an EPA approved laboratory for analysis of *Cryptosporidium* (and *E. coli*) samples for Long Term 2 Enhanced Surface Water Treatment Rule (LT2) compliance.

Sample Collection

Source Water – The EPA developed and validated Method 1623 for source water for Public Water Suppliers (PWSs) whose sources are surface water or groundwater under the direct influence of surface water (GWUDI).

Samples for analysis by Method 1623 can be collected as bulk ten-liter (10L) "grab" samples or field filtered (10 - 50L samples). Several types of filters have been approved by the EPA, including

the Filta-Max ™ and Filta-Max *xPress* filters (IDEXX Corp., Westbrook, ME), and the Envirochek™ and Envirochek HV filters (Pall Gelman Laboratory, Ann Arbor, MI). Larger sample volumes (50L) may yield more representative samples and field filtration does reduce shipping costs.

To simplify field filtration of 10L samples, ASI has developed *EasyVol*, a simple, volumetric system that eliminates the use of a flow totalizer, thus negating totalizer calibration and adjustment issues and improving sample volume accuracy measurement.

In areas with turbid waters, it may not always be possible to collect 10L using a single filter. For LT2 compliance, the sample volume analyzed must be at least 10L sample, or as much volume as two filters can accommodate before clogging, or 2 mL pf packed pellet volume, whichever comes first.

Finished Water – Although there are no current or pending regulations for finished water monitoring in the United States (and therefore no EPA approved procedures), Method 1623 can be applied to the detection of protozoa in finished water.

Sampling units and supplies for both bulk and field filtering of source and finished water, instructions and support are available from ASI.

Analytical Method

Bulk ("grab") samples are concentrated in the laboratory following the filter manufacturer's instructions. When samples are filtered (concentrated) in the field, laboratory procedures begin with filter elution.

Elution of captured organisms from the filter media is improved with Method 1623 compared to previous *Cryptosporidium* methods. For the Envirochek™ and Envirochek™ HV filters, a wrist-action shaker is used to physically agitate the capsule after partial filling with elution fluid. This action dislodges the particulates from the surface of the membrane filter, allowing them to backflushed out of the filter capsule by decanting the elution solution, which is subsequently concentrated by centrifugation.

After elution and concentration, the concentrate is then purified by IMS, which involves adding magnetic beads labeled with C*ryptosporidium* and *Giardia* specific antibodies to the sample concentrate and allowing the antibody-antigen reactions to bind the beads to the (oo)cysts. The sample is then magnetized, separating the (oo)cyst-magnetic bead complex from the sample debris, which is then discarded. The beads and (oo)cysts are then dissociated, the beads removed. The purified sample is then applied to a well slide, dried and stained with immunofluorescent antibody (IFA) stain and vital dyes. Examination is by fluorescence microscopy with confirmation by DAPI staining and DIC microscopy.

Method Approval and Recovery Efficiency

The EPA validates analytical methods when they have a regulatory mandate to do so. Method 1623 was developed and validated specifically for use in the Information Collection Rule Supplemental Survey (ICR-SS), and was conducted to augment the data generated from the ICR monitoring period regarding the occurrence of protozoa in source waters. Method 1623 has recently been revised and is included in the LT2 Rule by reference. The current is Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA; December 2005.[7]

Method 1623 is a performance based method and, as such, allows users to modify the method as improved technologies become available, as long as validation trials are conducted and specific performance criteria are achieved.[7] The Envirochek HV filters, used by ASI, were validated for source water in this manner, achieving EPA performance-based measurement system (PBMS) Tier 2 method validation requirements.[9]

Recently a modification of Method 1623 was released by EPA (1623.1). This procedure involves the addition of a pre-elution step on the filter and an additional rinse of the concentrated sample. Method 1623.1 is said to give improved recoveries of *Cryptosporidium*. ASI routinely performs both methods.

Some matrices can cause interference with these tests resulting in lower recoveries. ASI has developed an alternative methodology for such a matrix and offers custom-adapted methods for difficult matrices.

Matrix Spike (MS) Samples

In addition to analyst requirements and process controls, Method 1623 requires preparation and analysis of a matrix spike (MS) sample in conjunction with 1 in 20 samples from each source. [7]

A MS sample is collected in parallel with the corresponding monitoring sample and submitted to the laboratory where it is spiked with known quantities of protozoa to determine the analytical recovery efficiency. MS samples are an important quality assurance / quality control (QA/QC) aspect of Method 1623, and allow an assessment of whether the water from the site contains any substances that interfere with the analysis. This is particularly important in providing confidence in negative analytical results.

MS samples are site-specific and are distinct from laboratory QA/QC, which includes positive and negative staining controls and weekly Ongoing Precision and Recovery (OPR) samples.

Ideally, matrix spike (MS) samples are exact duplicates of the corresponding field samples; samples should be collected in parallel using a "Y" plumbing arrangement, although sequential sampling is acceptable. ASI recommends collection and submission of two 10L bulk water samples to ensure that the Field Sample and Matrix Spike Samples are treated identically. However, for LT2 compliance, the EPA allows the field sample to be field filtered and the MS sample to be submitted as a bulk sample. In addition, if large volumes samples (up to 50L) are collected, the EPA allows the MS sample to be partially field filtered (the last 10L must be submitted as a bulk sample for laboratory spiking). For example, a utility can collect a 50L field filtered sample with an MS sample which is 40L field filtered plus 10L bulk water). It should be noted that the volume of the Matrix Spike sample analyzed is required to be within 10% of the volume analyzed for the associated field sample.

In the lab, an MS sample is seeded with known amounts of *Giardia* and *Cryptosporidium* (using flow cytometry counted (oo)cysts) and then analyzed. The recovered number of *Giardia* and *Cryptosporidium* are then compared to the seeded quantities, and the recovery percentage is calculated. It should be noted that while Method 1623 includes "acceptance criteria" for recovery of protozoa from MS samples, there is no regulatory requirement in LT2 to meet these recoveries. The EPA recognizes that some water matrices contain substances that will interfere with Method 1623, and have stated that MS recoveries have no effect on data acceptability for LT2 compliance.

ColorSeed™

Although an improvement over the preceding method(s), the Matrix Spike remains less than optimal because it is only done periodically. Intermittent MS samples only determine the recovery efficiency in the given matrix at that time, and fail to reveal variation in recovery efficiency caused by different matrices or temporal variability in water quality.[10] Variability can be pronounced with surface water samples and more so with other matrices such as wastewater. Submitting a MS sample with every sample would enhance confidence in the data, but may be cost prohibitive.

Internal positive controls called ColorSeed™, (BioTechnology Frontiers, Pty Ltd., Sydney, Australia) are pre-stained *Cryptosporidium* and *Giardia* which are added to each sample (rather than to duplicate samples as in the MS approach) thus allowing an assessment of recovery efficiency for all samples vs. a 1 in 20 frequency as provided for by Method 1623. ColorSeed *Giardia* cysts and *Cryptosporidium* oocysts are labeled with a red dye such that they can be distinguished from indigenous organisms. ColorSeed (oo)cysts are gamma irradiated, flow cytometer enumerated and packaged in aliquots for single sample use. A vial of ColorSeed organisms is added to a water sample and the sample is otherwise processed in accordance with Method 1623. ColorSeed (oo)cysts behave in the same way as naturally occurring organisms in tests performed thus far. [10, 11] Therefore, the percent recovery of labeled (oo)cysts should be an accurate estimate of the recovery efficiency of indigenous protozoa. IPCs minimize the potential for false negative results and allow managers to make more accurate estimates of actual protozoa occurrence rates and concentrations.

Note: EPA Method 1623 has been validated only for use on raw (untreated) surface water

samples. Adapting the method to finished, ground or wastewater samples is a modified use of the method and is therefore not "validated". However, a very similar method has been used routinely in the UK for many years. Also, please note that ColorSeed is EPA approved for Method 1623 but not for use with LT2 compliance samples.

References

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