



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

Office of Ground Water and Drinking Water
Standards and Risk Management Division

September 30, 2010

MEMORANDUM

SUBJECT: Assessment of Protozoa Proficiency Testing Results from August 2010

FROM: Carrie Miller, *Cryptosporidium* Lab QA Evaluation Program Manager,
Technical Support Center *Carrie Miller*

TO: Laboratories Participating in Lab QA PT Program

EPA received data for *Cryptosporidium* and *Giardia* proficiency testing (PT) results from 57 laboratories for samples analyzed during the weeks of August 9 and August 23, 2010. Each laboratory received a set of 3 PT samples. The laboratories were instructed to add the samples to a volume of water (consistent with the sample volumes typically processed by the laboratory) and to analyze the samples like a field sample.

Sample Preparation and Distribution

The PT samples were prepared at the Wisconsin State Laboratory of Hygiene (WSLH) using a BD FacsAria flow cytometer to dispense the target number of organisms into 50-mL centrifuge tubes containing reagent water. Live *Cryptosporidium* oocysts were obtained from Sterling Parasitology Laboratory, Tucson, AZ. Live *Giardia* cysts were obtained from Waterborne™, New Orleans, LA. The spiked samples were shipped overnight to laboratories in coolers with ice packs.

WSLH prepared spiking suspensions on August 9 and August 23. The spike values used to calculate recovery were determined by calculating the means of the initial and ongoing calibration counts. These counts were acquired by directly sorting pre-stained cysts and oocysts onto well-slides for enumeration by epifluorescent microscopy. The target levels, calibration count means, and relative standard deviations (RSD) of these counts for each week are provided in **Table 1**. For each set of PT samples, all samples were spiked with both *Cryptosporidium* and *Giardia*. Diatomaceous earth was added to the suspension.

Table 1. Quality Control Counts of *Cryptosporidium* and *Giardia* Spiking Suspensions

Organism	Week	Number of calibration counts	Target Level	Mean Count	RSD (%)
<i>Cryptosporidium</i>	August 9	19	80	80.21	1.89
		15	95	94.80	1.07
	August 23	16	85	84.81	0.98
		21	90	89.71	1.01
<i>Giardia</i>	August 9	19	75	75.58	1.27
		16	80	80.81	1.82
	August 23	16	80	80.13	1.57
		19	85	84.84	1.38

The PT organisms were evaluated prior to generation of the test samples. Four replicate, blind spikes were prepared for different matrix combinations and processed using Method 1623. A qualitative assessment was also performed by fixing low-volume spikes on slides and evaluating the quality of the organisms and staining intensity. Results of these analyses were compared to historical PT results to ensure the organisms produced reliable results when used in Method 1623. PT samples were also analyzed during Week 2 of the August 2010 PT round to confirm that the organisms could be recovered at acceptable levels when used in Method 1623.

Results

All data from the PT samples and the associated ongoing precision and recovery (OPR) and method blank samples, including primary measurements, analyst information, holding times, and reagent lot numbers, were entered into a quality control (QC) database. The final concentrations and percent recoveries were automatically calculated from the primary measurements by the database. The database performed automated QC checks on holding times and results of the OPR and method blank samples to help ensure that Method 1622/1623 requirements were met. All of the WSLH spike preparation quality control was acceptable. Results of the screening and confirmation analyses demonstrated intact organism quality, brilliant FITC and DAPI fluorescence, and recoveries which met Method 1623 acceptance criteria and were comparable with past screens and rounds. All valid data were used to calculate the PT acceptance criteria of ± 2 standard deviations (SD) of the mean *Cryptosporidium* recovery for approved laboratories. The lower limit of the mean recovery was 15% and the upper limit was 91%. Summary statistics for method versions are provided in **Table 2**.

Table 2. Recovery Statistics for Each Method Version of Method 1623

Method Version	<i>Cryptosporidium</i> Recoveries				<i>Giardia</i> Recoveries			
	n	Mean (%)	Median (%)	RSD (%)	n	Mean (%)	Median (%)	RSD (%)
Envirochek HV™	132	51	54	45	126	49	52	41
Filta-Max®	30	59	60	22	27	60	59	20
Continuous Flow Centrifugation	3	75	74	9	3	60	61	7
Direct Centrifugation	3	57	57	5	3	58	58	14
Cryptonite	3	75	75	2	3	67	67	8
All versions	171	53	57	40	162	51	54	37

Evaluation of Laboratory Proficiency

The distribution of mean *Cryptosporidium* recoveries and relative standard deviations for each laboratory submitting valid data is presented by method version in **Figures 1 and 2**, respectively. If a laboratory performs poorly in a PT round, EPA may recommend additional follow-up action to demonstrate that the laboratory's performance remains acceptable. Additional actions may include submission of PT slides to EPA, repeat analyses, providing additional quality control data, and investigation of problems with reagents and equipment.

As detailed in the February 25, 2009 Federal Register Notice (<http://www.regulations.gov/fdmspublic/component/main?main=DocketDetail&d=EPA-HQ-OW-2002->

0011), a laboratory failing to submit valid PT results within the requested time period [10 business days after sample receipt] or meet PT acceptance limits for 2 out of 3 regular PT rounds may be downgraded or suspended for the analysis of *Cryptosporidium* under the Long Term 2 Enhanced Surface Water Treatment Rule.

Figure 1. Laboratory Mean Recoveries of *Cryptosporidium* by Method Version (Higher is Better)

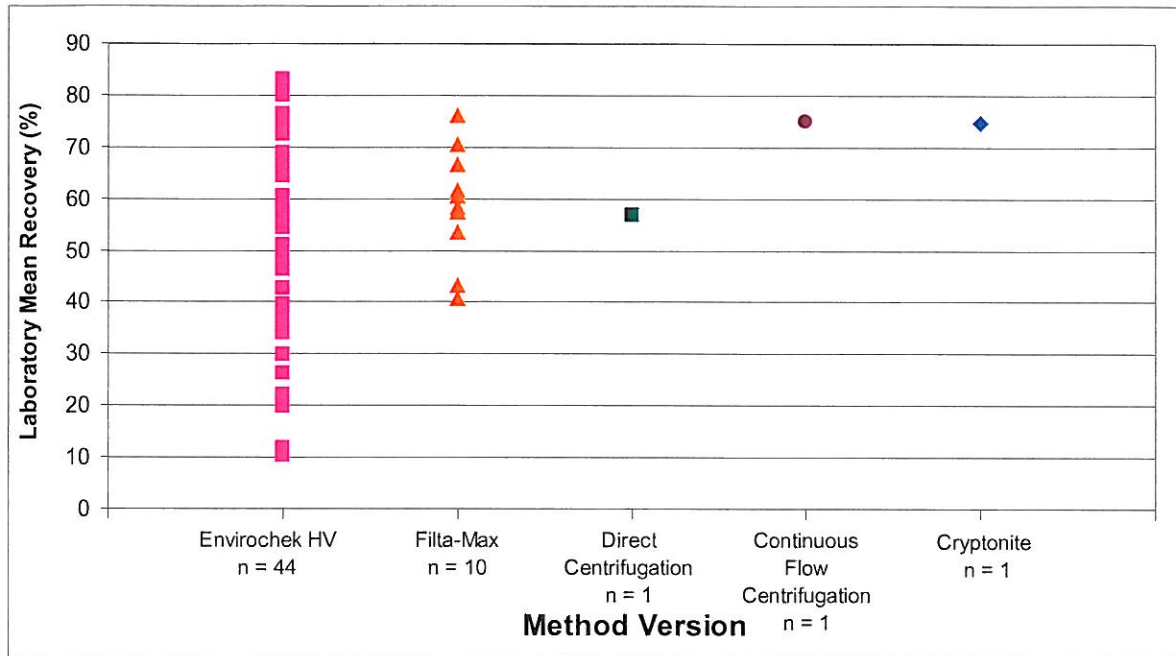


Figure 2. Laboratory Relative Standard Deviations by Method Version for *Cryptosporidium* Data (Lower is Better)

