

<sup>3</sup>If the system is a CWS or NTNCWSs and TTHM or HAA5 levels in the distribution system are at least 0.064 mg/L or 0.048 mg/L, respectively, calculated as an LRAA at any Stage 1 DBPR sampling site, then the system is triggered into disinfection profiling.

#### 4. Request for Comment

EPA requests comment on the reporting and recordkeeping requirements proposed for the LT2ESWTR.

Specifically, the Agency requests comment on the proposed requirement that systems report monthly on the use of microbial toolbox components to demonstrate compliance with their *Cryptosporidium* treatment requirements. An alternative may be for systems to keep records on site for State review instead of reporting the data.

#### K. Analytical Methods

EPA is proposing to require public water systems to conduct LT2ESWTR monitoring using approved methods for *Cryptosporidium*, *E. coli*, and turbidity analyses. This includes meeting quality control criteria stipulated by the approved methods and additional method-specific requirements, as stated later in this section. Related requirements on the use of approved laboratories are discussed in section IV.L, and proposed requirements for reporting of data were stated previously in section IV.J. EPA has developed draft guidance for sampling and analyses under the LT2ESWTR (see USEPA 2003g and 2003h). This guidance is available in draft form in the docket for today's proposal (<http://www.epa.gov/edocket/>).

##### 1. *Cryptosporidium*

a. *What is EPA proposing today?*  
Method 1622: "*Cryptosporidium* in Water by Filtration/IMS/FA" (EPA-821-R-01-026, April 2001) (USEPA 2001e) and Method 1623: "*Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (EPA 821-R-01-025, April 2001) (USEPA 2001f) are proposed for *Cryptosporidium* analysis under this rule. Methods 1622 and 1623 require filtration, immunomagnetic separation (IMS) of the oocysts from the captured material, and examination based on IFA, DAPI staining results, and differential interference contrast (DIC) microscopy for determination of oocyst concentrations.

##### Method Requirements

For each *Cryptosporidium* sample under this proposal, all systems must analyze at least a 10-L sample volume. Systems may collect and analyze greater than a 10-L sample volume. If a sample is very turbid, it may generate a large packed pellet volume upon centrifugation (a packed pellet refers to

the concentrated sample after centrifugation has been performed in EPA Methods 1622 and 1623). Based on IMS purification limitations, samples resulting in large packed pellets will require that the sample concentrate be aliquoted into multiple "subsamples" for independent processing through IMS, staining, and examination. Because of the expense of the IMS reagents and analyst time to examine multiple slides per sample, systems are not required to analyze more than 2 mL of packed pellet volume per sample.

In cases where it is not feasible for a system to process a 10-L sample for *Cryptosporidium* analysis (e.g., filter clogs prior to filtration of 10 L) the system must analyze as much sample volume as can be filtered by 2 filters, up to a packed pellet volume of 2 mL. This condition applies only to filters that have been approved by EPA for nationwide use with Methods 1622 and 1623—the Pall Gelman Envirochek™ and Envirochek™ HV filters, the IDEXX Filta-Max™ foam filter, and the Whatman CrypTest™ cartridge filter.

Methods 1622 and 1623 include fluorescein isothiocyanate (FITC) as the primary antibody stain for *Cryptosporidium* detection, DAPI staining to detect nuclei, and DIC to detect internal structures. For purposes of the LT2ESWTR, systems must report total *Cryptosporidium* oocysts as detected by FITC as determined by the color (apple green or alternative stain color approved for the laboratory under the Lab QA Program described in section VI.L), size (4–6 µm) and shape (round to oval). This total includes all of the oocysts identified as described here, less atypical organisms identified by FITC, DIC, or DAPI (e.g., possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.).

##### Matrix Spike Samples

As required by Method 1622 and 1623, systems must have 1 matrix spike (MS) sample analyzed for each 20 source water samples. The volume of the MS sample must be within ten percent of the volume of the unspiked sample that is collected at the same time, and the samples must be collected by splitting the sample stream or collecting the samples sequentially. The MS sample and the associated unspiked sample must be analyzed by the same procedure. MS samples must be spiked and filtered in the laboratory. However,

if the volume of the MS sample is greater than 10 L, the system is permitted to filter all but 10 L of the MS sample in the field, and ship the filtered sample and the remaining 10 L of source water to the laboratory. In this case, the laboratory must spike the remaining 10 L of water and filter it through the filter used to collect the balance of the sample in the field.

EPA is proposing to require the use of flow cytometer-counted spiking suspensions for spiked QC samples during the LT2ESWTR. This provision is based on the improved precision expected for spiking suspensions counted with a flow cytometer, as compared to those counted using well slides or hemacytometers. During the Information Collection Rule Supplemental Surveys, the mean relative standard deviation (RSD) across 25 batches of flow cytometer-sorted *Cryptosporidium* spiking suspensions was 1.8%, with a median of 1.7% (Connell *et al.* 2000). In EPA Performance Evaluation (PE) studies, the mean RSD for flow cytometer sorted *Cryptosporidium* spiking suspensions was 3.4%. In comparison, the mean RSD for *Cryptosporidium* spiking suspensions enumerated manually by 20 laboratories using well slides or hemacytometers was 17% across 108 rounds of 10-replicate counts.

QC requirements in Methods 1622 and 1623 must be met by laboratories analyzing *Cryptosporidium* samples under the LT2ESWTR. The QC acceptance criteria are the same as stipulated in the method. For the initial precision and recovery (IPR) test, the mean *Cryptosporidium* recovery must be 24% to 100% with maximum relative standard deviation (*i.e.*, precision) of 55%. For each ongoing precision and recovery (OPR) sample, recovery must be in the range of 11% to 100%. For each method blank, oocysts must be undetected.

Methods 1622 and 1623 are performance-based methods and, therefore, allow multiple options to perform the sample processing steps in the methods if a laboratory can meet applicable QC criteria and uses the same determinative technique. If a laboratory uses the same procedures for all samples, then all field samples and QC samples must be analyzed in that same manner. However, if a laboratory uses more than one set of procedures for *Cryptosporidium* analyses under LT2ESWTR then the laboratory must analyze separate QC samples for each