LABORATORY QUALITY ASSURANCE EVALUATION PROGRAM FOR ANALYSIS OF *CRYPTOSPORIDIUM* UNDER THE SAFE DRINKING WATER ACT

Information Collection Request:

Supporting Statement

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Ground Water and Drinking Water

April 2009

Table of Contents

Information Collection Request
Section 1: Part A of the Supporting Statement

1. Identification of the Information Collection

1(a) Title of the Information Collection

Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Renewal)

OMB Number: **2040 - 0246**
U.S. EPA Tracking Number: **2067.04**

1(b) Short Characterization

The U.S. Environmental Protection Agency (EPA) is requesting a renewal of the information collection request (ICR) for the Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Lab QA Program). This voluntary program applies to public and private laboratories that analyze water samples for *Cryptosporidium*. The program will help ensure that laboratories meet the quality assurance and quality control criteria of EPA Method 1622 and EPA Method 1623 (EPA, 2001a, 2001b) when using these methods for the determination of the identity and concentration of *Cryptosporidium* in source water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (IFA) microscopy.

Information collection activities required for laboratories seeking initial approval under the Lab QA Program include: a laboratory participation application; initial proficiency testing (IPT) results; a 2 day on-site evaluation of laboratory performance and data quality; and ongoing proficiency testing (OPT) results. All materials are being collected by the Office of Ground Water and Drinking Water (OGWDW). Information collection activities required for laboratories seeking continued approval under the Lab QA Program include: updating the application form; updating resumes for each staff member seeking EPA recognition under the program; standard operating procedures (SOPs); OPR and MS control charts; and training records for all analysts/technicians. A copy of the NELAC certificate (as applicable); participating in off-site re-evaluation activities, including submitting positive control and OPR slides, performing an independent count of a blind slide, performing internet analyst verification and characterizations; participating in a 1 day on-site evaluation; and analyzing OPT samples is also necessary. EPA estimates that a small subset of laboratories seeking continued recognition will have to perform follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results. This information collection will provide EPA with data to verify that the laboratories are capable of producing reliable data from the analysis of *Cryptosporidium* using EPA Method 1622 and EPA Method 1623.

The information collection will involve approximately 65 laboratories at a total cost of

approximately $411,729.40 or 4,843 labor hours annually. The total number of laboratories includes 63 laboratories that are currently approved and are anticipated to continue to seek approval under the Lab QA Program and 2 new State and/or EPA Regional laboratories per year that will seek initial approval under the Lab QA Program. The estimated total Agency burden, including contractual costs, is estimated at $564, 132.21.44 or 5,469 labor hours annually (Appendix L).

As of May 2007, EPA estimates that sufficient laboratory capacity exists for the LT2. EPA has generally postponed evaluation of additional laboratories, including commercial, county, municipal and utility laboratories, until further notice. EPA will consider evaluation of State and EPA Regional laboratories on a case-by-case basis, resources permitting, based on the role that States and EPA Regions play in the certification and approval programs for laboratories.

2. Need For and Use of the Collection

2(a) Need/Authority for the Collection

The information collection is needed by EPA to support the *Cryptosporidium* data gathering activities that are required under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). The Lab QA Program is being renewed because the *Cryptosporidium* laboratory evaluation program must continue for the duration of the LT2ESWTR. In addition, EPA proposed under the LT2ESWTR that drinking water plants monitoring their source waters for *Cryptosporidium* prior to rule implementation may apply to have these data "grandfathered." Renewing the Lab QA Program will help ensure that qualified laboratories are available to drinking water plants that are interested in pursuing this option.

2(b) Practical Utility/Users of the Data

Information collected under the Lab QA Program will be used by EPA to verify that *Cryptosporidium* occurrence data are generated by qualified laboratories that can perform the analyses acceptably. Use of qualified laboratories for source water monitoring by drinking water utilities will help ensure that the data collected are of known and reliable quality. Data quality could potentially be compromised in the absence of a program such as the Lab QA Program.

A list of laboratories that meet or continue to meet the evaluation program criteria is made available to the public at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html#listapprovedlabs and will provide a resource to aid drinking water utilities (and others interested in monitoring water for *Cryptosporidium* occurrence for the protection of public health) in selecting a qualified analytical laboratory.

3. Non-duplication, Consultations, and Other Collection Criteria

3(a) Non-duplication

The information requested from the respondents under this ICR is not available from other sources. The information requested will be used to assess the current ability of a laboratory to reliably analyze *Cryptosporidium* in water using EPA Method 1622 and EPA Method 1623. Information submitted for previous programs, such as the Information Collection Rule, is not applicable because older analytical methods were used and quality control requirements were different. The determination that this information is not available from other sources was made by the Office of Ground Water and Drinking Water Technical Support Center (TSC), which will be administering the Lab QA Program, and which has worked closely since 1996 with the community of capable laboratories that will be affected by this information collection.

3(b) Public Notice Required Prior to ICR Submission to OMB

On February 25, 2009 (74 FR 8529), EPA sought comments on this ICR pursuant to 5 CFR 1320.8(d). EPA received three comments during the comment period, which are addressed in the ICR.A copy of the first Federal Register notice is attached in Appendix D. Further details on the Lab QA Program have been added in Appendix G. The ICR was last renewed in 2005 (See Appendix C). EPA also has developed a webpage to provide further information on the program. The website can be accessed at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html.

3(c) Consultations

EPA conducted meetings with representatives of the drinking water treatment industry and the community of laboratories expected to seek EPA recognition under the Laboratory Quality Assurance Evaluation Program in Cincinnati, OH, on January 23 and March 12-13, 2001, and in Washington, DC, on February 13-14, 2001. EPA presented and discussed draft plans for the laboratory evaluation program at these meetings and sought input from the drinking water utility and laboratory representatives that attended these meetings.

Six laboratories were contacted in 2008/2009 for burden estimates for participating in the Lab QA Program. Each laboratory was asked to estimate the loaded cost of a manager/hr, a technician/hr, and an average cost for analyses of a Method1623 sample. The following laboratories have supplied burden estimates for participating in the Lab QA Program:

- ACT I Research and Analytical Laboratories
- CH Diagnostic and Consulting Services
- City of Los Angeles Department of Water and Power
- City of San Diego
- Lab/Cor, Inc
- Underwriters Laboratories, Inc.

EPA provides questionnaires to each laboratory after their on-site evaluation to provide feedback on the audit process and the auditors. EPA has used the feedback received from the laboratories

to improve the audit process.

3(d) Effects of Less Frequent Collections

Under the Lab QA Program, EPA requires laboratories to analyze single-blind OPT samples 3 times per year. This frequency enables EPA to independently verify that laboratories continue to perform in an acceptable manner. Less frequent OPT samples may not sufficiently capture a laboratory's performance over time. Laboratories are required to report OPT results within 14 days of sample receipt. Reporting OPT sample results at this frequency allows EPA to respond in a timely manner to any problems the laboratory may be having with analysis of *Cryptosporidium* in water.

3(e) General Guidelines

The Lab QA Program adheres to all of OMB's general guidelines for information collection.

3(f/g) Confidentiality/Sensitive Questions

The Lab QA Program does not ask any confidential or sensitive questions.

4. The Respondents and the Information Requested

4(a) Respondents/SIC Codes

The following is a list of SIC codes associated with laboratories affected by the requirements of this ICR:

8734 - Services: Testing Laboratories

4(b) Information Requested

(i) Data Items

- Laboratory participation application information
- Resumes for each staff member seeking EPA recognition under the program
- Standard operating procedures (SOPs)
- Ongoing precision and recovery (OPR) and matrix spike (MS) control charts
- Training records for all analysts/technicians added since the last audit
- Documentation of off-site re-evaluation activities including submitting positive control and OPR slides, performing an independent count of a blind slide, and performing internet analyst verification and characterizations
- Initial proficiency testing (IPT) data
- Ongoing proficiency testing (OPT) data
- Documentation of corrective actions taken in response to any deficiencies noted during the on-site evaluation

· Documentation of corrective actions taken in response to poor PT results

Maintain:
· IPT data
· OPT data

(ii) Respondent Activities

Activities for new State and/or EPA Regional laboratories seeking initial approval
· Completing initial laboratory participation application (1 time) (See Appendix F)
· Analyzing IPT samples (set of 8 samples, only 1 time) and reporting IPT data
· Analyzing OPT samples and reporting OPT data (set of 3 samples, only 2 times first year)
· Hosting on-site evaluation (1 time)

Activities for laboratories seeking to continue approval status
· Completing laboratory participation application package (1 time per 3 year period) (See Appendix G)
· Perform off-site re-evaluation activities (1 time per 3 year period)
· Analyzing OPT samples (set of 3 samples, 3 times per year) and reporting OPT data
· Hosting on-site evaluation (1 time per 3 year period)
· Perform follow-up action for poor PT results
· Perform and report 3 sets of OPTs for each additional method version

Response Activities/Year for 21 laboratories (63/3years) already approved and 2 new laboratories = (21 re-audit applications + 21 off-site activities + 21 on-site evaluations + (63 labs*3 OPTs) + (4 labs with additional method versions*3 OPTs) + 9 PT failures + 2 new applications + 2 New on-site evaluations + 2 IPTs + (2 Labs * 2 PTs))/65 = 4.4 Responses/year

5. The Information Collected - Agency Activities, Collection Methodology, and Information Management

5(a) Agency Activities

Agency activities associated with the OGWDW's Lab QA Program consist of the following:
·Reviewing initial laboratory participation applications and notifying laboratories of application status (1 time per laboratory per 3 year period)
·Reviewing laboratory re-evaluation applications and notifying laboratories of application status (1 time per laboratory per 3 year period)
·Conducting and reviewing off-site re-evaluation activities
·Preparing and distributing IPT samples; reviewing IPT data (1 time per new State and/or EPA Regional laboratory)
·Conducting on-site evaluations and re-evaluations of the laboratories seeking EPA recognition of laboratory capability and reporting on the results of these on-site evaluations (1 time per laboratory per 3 year period)
·Preparing and distributing OPT samples, which may include confounding organisms (2 times

first year only, 3 times per year, per laboratory, per method version); and tracking receipt of and reviewing OPT data and entering the data into a database

·Coordinate follow-up activities for poor PT results

5(b) Collection Methodology and Management

State and/or EPA Regional Laboratories Seeking Initial EPA Approval

State and EPA Regional laboratories interested in obtaining initial EPA recognition of their capability to perform analyses using EPA Method 1622 and EPA Method 1623 may submit applications to EPA, but should contact the laboratory approval manager prior to investing substantial effort towards their application. A decision by the Agency to review an application, to send initial PT samples, and/or to schedule or conduct an on-site evaluation and data evaluation, does not ensure that the review process will be completed or that the laboratory will ultimately be approved. Decisions will be made based on the facts associated with a particular application and actions will be taken as Agency resources permit. Subject to availability of resources, EPA will evaluate the applications for completeness and compare the information to the recommended criteria specified in the Federal Register Notice. The criteria include:

1. Recommended personnel criteria:

Principal Analyst/Supervisor (at least 1 per laboratory) should have:

- BS/BA in microbiology or closely related field
- A minimum of 1 year of continuous bench experience with *Cryptosporidium* and IFA microscopy
- A minimum of six months experience using EPA Method 1622 and/or EPA Method 1623
- A minimum of 100 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 50 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method (EPA, 1996)) for the specific analytical procedure they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed principal analyst/supervisor

Other Analysts (no minimum number of analysts per laboratory) should have:

- Two years of college (or equivalent) in microbiology or closely related field
- A minimum of six months of continuous bench experience with *Cryptosporidium* and IFA microscopy
- A minimum of 3 months experience using EPA Method 1622 and/or EPA Method 1623
- A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method) for the specific analytical procedures they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed other analysts

Technician(s) (no minimum number of technicians per laboratory) should have:

- Three months experience with the specific parts of the procedure they will be performing
- A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method) for the specific analytical procedures they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed technician(s)

2. Recommended laboratory criteria:

- Appropriate instrumentation as described in EPA Methods 1622 and 1623 (EPA, 2005)
- Equipment and supplies as described in EPA Methods 1622 and 1623 (EPA 2005)
- Detailed laboratory standard operating procedures for each version of the method that the laboratory will use to conduct the *Cryptosporidium* analyses
- Laboratory should provide a current copy of the table of contents of their laboratory's quality assurance plan for protozoa analyses
- EPA Method 1622 or EPA Method 1623 initial demonstration of capability (IDC) data, which include precision and recovery (IPR) test results and matrix spike/matrix spike duplicate (MS/MSD) test results for *Cryptosporidium*. EPA intends to evaluate the IPR and MS/MSD results against the performance acceptance criteria in the 2005 version of EPA Method 1622 or EPA Method 1623 (EPA, 2005).

  During on-site evaluations, EPA will evaluate laboratories' performance of the methods, as well as laboratories' data recording and quality control practices using standardized checklists.

  IPT and OPT data will be reviewed against the requirements of Method 1622/1623 and the recommended criteria specified in Federal Register Notice (Appendix D). Data for the IPT and OPT samples will be entered into and stored in a QC database with automated data review and calculation functions. Automating data review functions reduces resources required for data review and ensures that all samples are reviewed in a consistent manner.

Laboratories Seeking Continued EPA Approval

Laboratories seeking continued EPA recognition of laboratory capability to perform analyses using EPA Method 1622 and EPA Method 1623 will have demonstrated, and are to continue to demonstrate, proficient and reliable detection and enumeration of *Cryptosporidium* in surface water sources for public water systems. They will have passed all elements in the Lab QA Program and continue to successfully participate in all program activities. Approved laboratories are responsible for notifying EPA of losses of key personnel or essential equipment and changes in policies or procedures that directly affect the validity of data or any other change affecting the capability of the laboratory including change in location. Participating laboratories are to also demonstrate ongoing capability and method performance by following all applicable method quality control (QC) procedures, analyzing ongoing proficiency testing (PT) samples (3 times per year), submitting requested data to EPA, and participating in periodic re-evaluations.

The Lab QA Program procedures have been updated to reflect that the minimum recovery for *Cryptosporidium* in ongoing precision and recovery (OPR) samples is now 22 percent (See Appendix K). Laboratories are to document a minimum of 22 percent recovery for OPR samples in an updated QC chart prior to analysis of LT2ESWTR samples at the frequency required in Section 9.7 of the method. If a laboratory submits poor PT results, 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 QC data, and investigation of problems with reagents and

equipment. Repeated failure to demonstrate laboratory capability and acceptable method performance may result in suspension or downgrading of approval status as outlined in "Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* Under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (See Appendix J)." Provided EPA has sufficient resources to review requests for upgrade or reinstatement, laboratories may have to undertake additional activities such as analyzing additional PT samples, undergoing an on-site evaluation, and/or counting blind spiked slides in order to have their status upgraded or their approval reinstated.

EPA may re-evaluate laboratories participating in the program to verify *Cryptosporidium* laboratory quality assurance (QA) on both an "as-needed" and periodic basis (generally not exceeding once every 3 years). This process is detailed in Appendix G. In the case of a periodic assessment, EPA will generally notify the laboratory that they are due for re-evaluation and request a package with documentation of personnel status, equipment maintenance, standard operating procedures, training records, and QC charts. After the package has been received, it will be evaluated for completeness. EPA generally contacts the laboratory within 15 days of package submission if information is missing. When a complete package has been received, the following steps will complete the process:

1. The laboratory will send positive staining control and OPR slides for evaluation by EPA (Appendix G).

2. The laboratory will order blind slides spiked with *Cryptosporidium* from a qualified vendor for each analyst. Each analyst will perform an independent count of 1 slide. The results and slides will be submitted to a technical auditor (Appendix G).

3. EPA will schedule an on-line Internet analyst verification of performance for microscopists to demonstrate their ability to identify *Cryptosporidium* oocysts (Appendix I).

4. EPA conducts a 1-day on-site evaluation that will primarily focus on method performance and data recording. Laboratory personnel will be asked to order blind oocyst suspensions for spiking reagent water and an IMS control in the presence of an auditor, and then complete the analyses within applicable method holding times and send results to EPA (Appendix G).

5. EPA will send the laboratory a report detailing all findings, generally within 60 days after the evaluation is complete. The laboratory is then asked to provide written responses to any deficiencies identified in the report within 60 days. Provided all responses to the deficiencies cited in the report are acceptable, the Lab QA Program will then base its decision for continued laboratory approval on PT results, quality of the positive control and OPR slide, slide counts, Internet analyst verification, on-site evaluation and recovery values for blind analyses initiated during the on-site evaluation (See Appendices G, H, and I).

5(c) Small Entity Flexibility

The Lab QA Program is a voluntary program; any entity that believes this program will impose undue burden is not required to participate in the Lab QA Program.

Small businesses are defined as any business that is independently owned and operated and not dominant in its field as defined by the Small Business Administration (SBA) regulations under Section 3 of the Small Business Act.

Small businesses may opt to seek EPA recognition of laboratory capability to perform *Cryptosporidium* water analyses using only 1 version of EPA Method 1622 or EPA Method 1623, as opposed to being evaluated for multiple versions, and reduce the burden associated with participation in the Lab QA Program.

5(d) Collection Schedule

The Lab QA Program is a voluntary program. No laboratories are required to participate or submit any information.

EPA will consider evaluation of State and EPA Regional laboratories on a case-by-case basis, resources permitting, based on the role that States and EPA Regions play in the certification and approval programs for laboratories. Laboratories that are currently participating in the program and have already been evaluated during the original ICR time frame may be asked to submit an updated application and be re-evaluated during the period for this ICR renewal. Laboratories that have successfully completed the audit process will not be evaluated more than once per 3 year period.

The Office of Ground Water and Drinking Water has prepared a document, "Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* Under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR)," to describe how the program generally assesses the ongoing capability of laboratories approved by EPA to perform *Cryptosporidium* analyses in support of the Long Term 2 Enhanced Surface Water Treatment Rule. EPA has been providing technical assistance to laboratories to improve their standard operating procedures for documentation of quality control, on-going precision and recovery of spiked organisms, and proficiency test performance since the inception of the program. As the Lab QA Program has matured and several years of quality control data have been collected, the practices and procedures have been refined and developed them into a protocol, modeled after the EPA's Drinking Water Laboratory Certification Program, for ensuring reliable detection of microorganisms.

6. Estimating the Burden and Cost of the Collection

6(a) Estimating Respondent Burden

Below are summaries of respondent burden hours for this information collection. EPA consulted with fewer than nine respondents from the community of laboratories that have voluntarily applied for EPA approval of laboratory capability to perform *Cryptosporidium* analyses using EPA Method 1622 and EPA Method 1623 to obtain burden hour estimates. For specific burden breakdowns, refer to the *Participating Laboratories Seeking Continued Approval* and *Laboratories Seeking Initial Approval* burden tables in Appendix L. The burden estimates were also addressed in the Federal Register Notice published in February 2009 (See Appendix D).

EPA estimates that 63 laboratories will continue to participate in the EPA Lab QA Program, of which 28 are private and 35 are state or local government entities. EPA estimates that there will be an additional 2 State and/or EPA Regional laboratories per year seeking initial EPA approval under the Lab QA Program, for a total of 65 laboratories.

Activities for Laboratories Seeking Continued Approval

Laboratories seeking continued approval under the Lab QA Program will complete an application package, including: 1) completing the application form; 2) providing resumes for each staff member seeking EPA recognition under the program; 3) standard operating procedures (SOPs); 4) OPR and MS control charts; 5) training records for all analysts/technicians added since the last audit; 6) a copy of NELAC certificate (as applicable) as documentation of equipment maintenance. The deadline for application submission is 60 days from the request receipt. Since laboratories have to submit the application only 1 time per 3 year period, the number of laboratories expected to submit applications were evenly distributed over a 3 year-period to estimate burden hours and costs per year (e.g., laboratories seeking continued approval, 63 laboratories/3 years = approximately 21 labs/year). Burden hours associated with submitting the completed application package for the laboratories applying for continued EPA recognition are estimated at 336 labor hours per year.

Laboratories seeking continued approval will complete off-site re-evaluation activities including submitting positive control and OPR slides, performing an independent count of a blind slide, and performing internet analyst verification and characterizations. Since laboratories only have to complete off-site re-evaluation activities 1 time per 3 year period, the number of laboratories expected to complete off-site re-evaluation activities were evenly distributed over a 3 year-period to estimate burden hours per year (e.g., laboratories seeking continued approval, 63 laboratories/3 years = approximately 21 labs/year). Burden hours associated with completing off-site re-evaluation activities for the laboratories applying for continued EPA recognition are estimated at 357 labor hours per year.

Each laboratory seeking continued EPA recognition of laboratory capability under the Lab QA Program will participate in a 1 day on-site evaluation. The burden hours associated with this task include time required to attend short briefings by the auditors before and after the audit, demonstrate the techniques for the methods for which they are seeking EPA recognition, participate in discussions with the auditors, and respond to any deficiencies noted in the audit report. Laboratories will purchase blind spikes from a qualified vendor and will process samples to demonstrate method performance. Because laboratories will only undergo an on-site evaluation 1 time every 3 years, the number of laboratories expected to be evaluated were evenly distributed over a 3 year period to estimate burden hours per year (e.g., laboratories seeking continued approval, 63 laboratories/3 years = approximately 21 labs/year). Burden hours for all laboratories applying for continued recognition are estimated at 651 labor hours per year (Appendix L).

Laboratories approved to participate in the program will analyze a set of OPT samples, which may include confounding organisms (3 samples per set) 3 times a year for each method version for which they are seeking EPA recognition. The burden estimates associated with this task include labor associated with analyzing samples and documenting the data for each OPT set. Burden hours for all laboratories seeking continued recognition and analyzing 1 set of OPT samples 3 times a year are estimated at 2867 labor hours per year (Appendix L). It is estimated that 4 laboratories seeking continued recognition under the Lab QA Program will perform more than 1 method version and will perform an additional set of PT samples for each additional method version 3 times a year. Burden hours for laboratories seeking continued recognition and performing an additional set of PT samples for each additional method version are estimated at 182 labor hours per year (Appendix L). EPA estimates that a small subset of laboratories seeking continued recognition will have to perform follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results. Burden hours for laboratories seeking continued recognition and performing follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results are estimated at 216 labor hours per year (Appendix L).

Activities for State and/or EPA Regional Laboratories Seeking Initial Approval

State and/or EPA Regional laboratories seeking initial approval under the Lab QA Program will have to complete an application package that requires the following: 1) completing the application form and a self-audit checklist; 2) providing resumes for each staff member seeking EPA recognition under the program; 3) providing copies of existing laboratory procedures for each version of the method for which the laboratory is seeking EPA recognition; and 4) providing the results of initial demonstration of capability data for each version of the method for which the laboratory is seeking EPA recognition. There is not a deadline for application submission, and laboratories can

submit applications at any time. Since laboratories only have to submit the application 1 time, the number of laboratories expected to submit applications over a 3 year period were evenly distributed over that period to estimate burden hours per year (e.g., laboratories seeking initial approval, 6 laboratories/3 years = approximately 2 labs/year). Burden hours associated with submitting the completed application package for the laboratories applying for EPA recognition are estimated at 32 labor hours per year (Appendix L).

Each new State and/or EPA Regional laboratory seeking initial EPA recognition of laboratory capability under the Lab QA Program will undergo an on-site evaluation. Laboratories seeking initial recognition will participate in a 2 day on-site process. The burden hours associated with this task include time required to attend short briefings by the auditors before and after the audit, demonstrate the techniques for the methods for which they are seeking EPA recognition, participate in discussions with the auditors, and respond to any deficiencies noted in the audit report. Because laboratories will only undergo an initial on-site evaluation 1 time, the number of laboratories expected to be evaluated were evenly distributed over a 3-year period to estimate burden hours per year (e.g., laboratories seeking initial approval = 6 laboratories/3 years = approximately 2 labs/year). Burden hours associated with the on-site evaluation for all laboratories applying for initial EPA recognition are estimated at 70 labor hours per year (Appendix L).

Each new State and/or EPA Regional laboratory will analyze a set of IPT samples, (8 samples per set) for the version of the method for which they are seeking EPA recognition. The burden for this task includes labor associated with the analyzing samples and documenting the data for each IPT set. Since laboratories have to analyze a set of IPT samples only once, the number of laboratories expected to analyze IPT samples were evenly distributed over a 3-year period to estimate burden hours per year (e.g., laboratories seeking initial approval = 6 laboratories/3 years = approximately 2 labs/year). Laboratories that are already participating in the Lab QA Program will not have to repeat their IPT analysis and are not included in the burden estimates. Burden hours for all laboratories analyzing IPT samples are estimated at 72 labor hours per year (Appendix L).

During the first year of participation in the Lab QA Program, new State and/or EPA Regional laboratories will analyze 2 sets of OPT samples (after completing IPT analysis). The burden for this task includes labor associated with the analyzing samples and documenting the data for each OPT set. Burden hours for all laboratories seeking initial recognition and analyzing 1 set of OPT samples 2 times a year for the first year are estimated at 60 labor hours per year (Appendix L).

6(b) Estimating Respondent Costs

Below are summaries of respondent burden costs for this information collection. EPA consulted with fewer than nine respondents from the community of laboratories that have voluntarily applied for EPA approval of laboratory capability to perform *Cryptosporidium* analyses using EPA Method 1622 and EPA Method 1623 to obtain burden cost estimates. Respondent costs associated with analysis of PT samples include labor and O&M costs, which are estimated at $188 per analytical sample. For specific burden breakdowns, refer to the *Participating Laboratories Seeking Continued Approval* and *Laboratories Seeking Initial Approval* burden tables in Appendix L.

EPA estimates that 63 laboratories will continue to participate in the EPA Lab QA Program. EPA estimates that there will be an additional 2 State and/or EPA Regional laboratories per year seeking initial EPA approval under the Lab QA Program, for a total of 65 laboratories.

Cost for Laboratories Seeking Continued Approval

Burden costs associated with submitting the completed application package for the laboratories applying for continued EPA recognition are estimated at $19,320.00 per year. Burden costs associated with this task include $19,005.00 for labor and $315.00 for Operations and Maintenance (O&M) (Appendix L).

Burden costs associated with completing off-site re-evaluation activities for the laboratories applying for continued EPA recognition are estimated at $27,111.00 per year. Burden costs associated with this task include $19,761.00 for labor and $7,350.00 for O&M (Appendix L).

Burden costs for all laboratories participating in the 1-day on-site evaluation are estimated at $48,510.00 per year (Appendix L). Burden costs associated with this task include $38,010.00 for labor and $10,500.00 for O&M (Appendix L).

Burden costs for all laboratories analyzing 1 set of OPT samples 3 times a year are estimated at $264,360.60 per year. Burden costs associated with this task include $157,764.60 for labor and $106,596.00 for O&M (Appendix L). It is estimated that 4 laboratories seeking continued recognition under the Lab QA Program will perform more than 1 method version and will perform an additional set of PT samples for each additional method version 3 times a year. Burden costs for laboratories performing an additional set of PT samples for each additional method version are estimated at $16,784.80 per year. Burden costs associated with this task include $10,016.80 for labor and $6,768.00 for O&M (Appendix L). A small subset of laboratories seeking continued recognition will have to perform follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results. Burden costs for laboratories performing follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results are estimated at $17,109.00 per year. Burden costs associated with this task include $12,033.00 for labor and $5,076.00 for O&M (Appendix L).

Cost for State and/or EPA Regional Laboratories Seeking Initial Approval

Burden costs associated with submitting the completed application package for the State and/or EPA Regional laboratories applying for EPA recognition are estimated at $1,840.00 per year (Appendix L). Burden costs associated with this task include $1,810.00 for labor and $30.00 for O&M (Appendix L).

Burden costs associated with the on-site evaluation for State and/or EPA Regional laboratories applying for initial EPA recognition are estimated at $4,216.00 per year (Appendix L). Burden costs associated with this task include $4,186.00 for labor and $30.00 for O&M (Appendix L).

Burden costs for State and/or EPA Regional laboratories analyzing 1 set of IPT samples are estimated at $6,916.00 per year (Appendix L). Burden costs associated with this task include $3,908.00 for labor and $3,008.00 for O&M (Appendix L).

Burden costs for State and/or EPA Regional laboratories analyzing 1 set of OPT samples 2 times a year for the first year are estimated at $5,562.00 per year. Burden costs associated with this task include $3,306.00 for labor and $2,256.00 for O&M (Appendix L).

6(c) Estimating Agency Burden and Costs

Below are Agency burden hours and associated financial costs pertaining to implementation of the Lab QA Program. For a specific breakdown of burden hours and financial costs, refer to the *Agency Burden* table in Appendix L. Costs and burden hours are broken out based on activities completed by the Agency and supporting contractors. Based on the 2009 GS schedule for the Cincinnati area and the standard government benefits multiplication factor of 1.6, EPA estimates an average hourly cost of $ 101.21 /hour for Agency legal staff, $ 80.93 /hour for Agency program management staff, and $ 19.68 /hour for Agency clerical staff. Based on the published schedule of contractor labor rates for the years covered by this program, the average loaded burden hours and costs for contractor labor were estimated at $119.00/hour for expert staff, $102.00/hour for management staff, and $86.00/hour for technical staff.

Agency burden is estimated based on the labor hours associated with performing each task per laboratory seeking laboratory capability recognition. To get the total annual cost, hours and costs are then multiplied by the estimated number of respondents and added to the capital and O&M costs. The burden associated with each information collection task is shown in a separate row of the burden table. EPA estimates that 63 laboratories will continue to participate in the EPA Lab QA Program. EPA estimates that there will be an additional 2 laboratories per year seeking initial EPA approval under the Lab QA Program, for a total of 65 laboratories.

The Agency will review the laboratory participation applications to ensure that all the required information has been submitted and that each laboratory applicant has the necessary experience and qualifications to acceptably analyze water samples for *Cryptosporidium*. The labor hours and costs associated with this task include reviewing the initial laboratory application or re-evaluation application and notifying the laboratory if their application is acceptable or requires submission of additional information. Each new State and/or EPA Regional laboratory will be required to submit an initial application, while continuing laboratories will send a renewal application every 3 years, the number of laboratories expected to submit applications is evenly distributed over 3 years in order to determine labor hours and costs per year. The Agency burden associated with review of initial laboratory participation applications is estimated at 20 labor hours and a cost of $1,699.72 per year. The Agency burden associated with review of re-evaluation applications is estimated at 357 labor hours and a cost of $30,048.06 per year.

Because the Agency developed a re-evaluation process to reduce the amount of time on-site at laboratories, the Agency will conduct off-site activities, to include remote analyst verification, positive slide control and ongoing precision and recovery (OPR) slide evaluation, and equipment evaluations. The Agency burden associated with off-site activities is estimated at 1092 labor hours and a cost of $92,833.65 per year.

The Agency will perform initial on-site evaluations and re-evaluations of each laboratory to determine if the laboratory has the required equipment and facilities, has an appropriate QC program in place, and is performing the method properly. Labor hours and costs include scheduling the on-site evaluation, travel, conducting the evaluation, documenting the results of the evaluation, notifying the laboratory of the results of their evaluation, and tracking the progress and costs of these activities. The Agency burden associated with performing initial on-site evaluations is estimated at 288.5 labor hours per year and a cost of $35,347.91 per year. The Agency burden associated with performing on-site re-evaluations is estimated at 2315 labor hours per year and a cost of $287,993.00 per year.

To test the ability of the laboratory to acceptably analyze water samples for *Cryptosporidium*, the Agency will distribute IPT samples to new State and/or EPA Regional laboratories seeking initial approval under the Laboratory Quality Assurance Evaluation Program. The labor hours and costs associated with this task include notifying laboratories when they will receive their samples, preparing the samples, and shipping the samples to the laboratories. The capital startup costs associated with preparing the proficiency testing samples are included in the costs of preparing the IPT samples. The Agency will review the IPT data submitted by the laboratory to verify that the data submission is complete, the method requirements were met, and that the laboratory's performance was acceptable. After the review is complete, the Agency will notify the laboratory whether their performance on the IPT samples was acceptable. Because each laboratory will be required to analyze IPT samples only once, the number of laboratories expected to analyze IPT samples is evenly distributed over 3 years in order to determine labor hours and costs per year. The Agency burden associated with IPT samples is

estimated at 18 labor hours per year and a cost of $2,191.40 per year.

To test the ability of the laboratory to acceptably analyze water samples for *Cryptosporidium* on an on-going basis, the Agency will distribute OPT samples to the laboratories participating in the Laboratory Quality Assurance Evaluation Program. The labor hours and costs associated with this task include notifying laboratories when they will receive their next samples, preparing the samples, and shipping the samples to the laboratories. The capital startup costs associated with preparing the proficiency testing samples are included in the costs of preparing the OPT samples. On an ongoing basis, the Agency will review OPT data submitted by the laboratories to verify that the data submission is complete, the method requirements were met, and that the laboratories' performance was acceptable. The labor hours and costs associated with reviewing these data include data entry into the QC database, automated data review, and notification of the laboratory regarding the results. The Agency burden associated with OPT samples is estimated at 1273 labor hours per year and a cost of $104,516.65 per year. The Agency burden associated with preparing and distributing spiking suspensions for OPTs for initial labs and reviewing OPT data is estimated at 24 labor hours per year and a cost of $2,645.08 per year.

Laboratories with poor PT results may be asked to perform additional actions to demonstrate proficiency. EPA may review PT slides, review repeat analysis data, review additional quality control data, and review documentation of reagents and equipment investigation. The Agency burden associated with providing technical support to laboratories with poor PT results is estimated at 81 labor hours per year and a cost of $6,856.74 per year.

6(d) Estimating the Respondent Universe and Total Burden and Costs

The affected entities include public and private water testing laboratories. EPA estimates that 63 laboratories will continue to participate in the EPA Lab QA Program, of which 28 are private and 35 are state or local government entities. EPA estimates that there will be an additional 2 State and/or EPA Regional laboratories per year seeking initial EPA approval under the Lab QA Program, for a total of 65 laboratories. The respondent total burden and cost are provided in the *Total Respondent and Agency Burden Tables* in Appendix L and are described in greater detail in Sections 6(a) - 6(c).

6(e) Bottom Line Burden Hours and Cost Tables

(i) Respondent Tally

Refer to the burden table in Appendix L titled, *Total Respondent and Agency Burden Tables*, for a specific breakdown of the respondent costs. The Lab QA Program will affect approximately 65 respondents. The respondents will engage in 4.4 different tasks (refer to Section 4(b)(ii)) involving 4,843 labor hours and costing approximately $269,800.40 per year for labor (Appendix L). Respondents will invest $0.00 per year in

capital/start-up costs and $141,929.00 per year in O&M costs (Appendix L).

(ii) Agency Tally

Refer to the burden table in Appendix L titled, *Total Agency and Agency Burden Tables*, for a summary of Agency costs. Nine Agency tasks are associated with the Lab QA Program. These tasks will involve approximately 5,469 labor hours annually resulting in a cost of $486,347.21 per year for labor. The Agency will invest approximately $0.00 per year in capital/start-up costs and $77,785.00 per year in O&M costs.

6(f) Reasons for Change in Burden

Changes in burden have occurred due to inflation, re-evaluation of hours for tasks, and improved demonstration of capability. Inflation has increased all operation and maintenance and labor costs accordingly. The increase in the respondent universe has increased the overall burden costs for the respondents. EPA's original estimates for hours to participate and maintain the Lab QA Program were made before the program began. Because the program has matured and several years of QC data have been collected, the burden has changed for performing improved and refined procedures. The burden for some tasks has been estimated and will be re-evaluated as the program progresses. EPA has included the above section entitled "Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* Under the Long Term 2 Enhanced Surface Water Treatment Rule." Some approved laboratories may have to undertake additional activities to demonstrate continued acceptable performance to EPA, which may increase the burden of participation in the Lab QA Program for those laboratories. EPA estimates that 9 laboratories per year may have to undertake additional activities to demonstrate acceptable performance to EPA. These estimates will be corrected as the program continues.

6(g) Burden Statement

The annual reporting and recordkeeping burden for this collection is estimated to average 74.5 hours annually per laboratory (the combined total hours per year for laboratories seeking initial approval and laboratories seeking continued approval divided by 65 laboratories).

Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of

information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations are listed in 40 CFR Part 9 (http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr92_main_02.tpl) and 48 CFR Chapter 15 (http://www.access.gpo.gov/nara/cfr/waisidx_00/48cfrv6_00.html).

To comment on the Agency's need for this information, the accuracy of the provided burden estimates, and any suggested methods for minimizing respondent burden, including use of automated collection techniques, EPA has established a public docket for this ICR under Docket ID Number EPA-HQ-OW-2002-0011 which is available for online viewing at www.regulations.gov, or in person viewing at the Water Docket in the EPA Docket Center (EPA/DC), EPA West, Room 3334, 1301 Constitution Avenue, NW, Washington, D.C. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Reading Room is (202) 566-1744, and the telephone number for the Water Docket is (202) 566-2426. An electronic version of the public docket is available at www.regulations.gov. This site can be used to submit or view public comments, access the index listing of the contents of the public docket, and to access those documents in the public docket that are available electronically. When in the system, select "search," then key in the Docket ID Number identified above. Also, you can send comments to the Office of Information and Regulatory Affairs, Office of Management and Budget, 725 17th Street, NW, Washington, D.C. 20503, Attention: Desk Officer for EPA. Please include the EPA Docket ID Number EPA-HQ-OW-2002-0011 and OMB Control Number 2040-0246 in any correspondence.

APPENDIX A

Federal Register Notice:
Laboratory Quality Assurance Evaluation Program/
Information Collection Request
[Published December 29, 2000]

ENVIRONMENTAL PROTECTION AGENCY

[FRL- 7152-6]

Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act; Agency Information Collection: Proposed Collection; Comment Request

AGENCY: Environmental Protection Agency.

ACTION: Notice; Request for Comment.

SUMMARY: Today's notice invites comment on the U.S. Environmental Protection Agency's (EPA's) proposed Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Lab QA Program) (Section I). EPA also plans to submit to the Office of Management and Budget (OMB) for review and approval an Information Collection Request (ICR) associated with information collections under the proposed Lab QA Program (Section II). EPA is requesting comments on specific aspects of the proposed Lab QA Program and the ICR. Finally, EPA solicits comments on its intention to seek an emergency clearance from OMB to begin collecting data from laboratories that are interested in participating in the Lab QA Program prior to OMB's final approval of the ICR.

DATES: The Agency requests comments on today's notice. Comments must be received or post-marked by midnight May 3, 2002. If EPA does not receive adverse comments on or before April 3, 2002 regarding EPA's request for an emergency clearance, the Agency intends to seek a 90-day emergency clearance from OMB to begin collecting data from laboratories that are interested in participating in the Lab QA Program.

ADDRESSES: Please send an original and three copies of your written comments and enclosures (including references) to the W-01-17 Comment Clerk, Water Docket (MC-4101), EPA, 1200 Pennsylvania Avenue, NW, Washington, DC 20460. Due to the uncertainty of mail delivery in the Washington, DC area, in order to ensure that all comments are received please send a separate copy of your comments via electronic mail (e-mail) to Mary Ann Feige, EPA, Office of Ground Water and Drinking Water, feige.maryann@epa.gov, or mail to the attention of Mary Ann Feige, EPA, Technical Support Center, 26 West Martin Luther King Drive (MS-140), Cincinnati, Ohio 45268. Hand deliveries should be delivered to: EPA's Water Docket at 401 M Street, SW, Room EB57, Washington, DC 20460. Please make certain to reference EPA ICR No. 2052.02 and OMB Control No. 2040-0229.

FOR FURTHER INFORMATION: For a copy of the ICR, contact Sharon Gonder at EPA by phone at (202) 564-5256 or by email at gonder.sharon@epa.gov or download off the Internet at http://www.epa.gov/icr and refer to EPA ICR No. 2052.02. For technical inquiries, contact Mary Ann Feige, EPA, Office of Ground Water and Drinking Water, Technical Support Center, 26 West Martin Luther King Drive (MS-140), Cincinnati, Ohio 45268, fax number, (513) 569-7191, e-mail address, feige.maryann@epa.gov.

SUPPLEMENTARY INFORMATION:

Submission of comments.

Individuals who want EPA to acknowledge receipt of their comments should enclose a self-addressed, stamped envelope. No facsimiles (faxes) will be accepted. Comments may also be submitted electronically to ow-docket@epamail.epa.gov.

Electronic comments must be submitted as an ASCII, WP5.1, WP6.1 or WP8 file avoiding the use of special characters and form of encryption. Electronic comments must be identified by docket number W-01-17. Comments and data will also be accepted on disks in WP5.1, 6.1, 8 or ASCII file format. Electronic comments on this notice may be filed online at many Federal Depository Libraries.

Availability of docket.

The record for this notice has been established under docket number W-01-17, and includes supporting documentation as well as printed, paper versions of electronic comments. The record is available for inspection from 9 a.m. to 4 p.m., Monday through Friday, excluding legal holidays at the Water Docket, EB 57, EPA Waterside Mall, 401 M Street, SW, Washington, DC 20460. For access to docket materials, please call (202) 260-3027 to schedule an appointment.

Section I: Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act

In September 2000, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee (Committee) signed an Agreement in Principle (Agreement) (65 FR 83015, Dec. 29, 2000) (EPA, 2000) with consensus recommendations for two future drinking water regulations: the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectants and Disinfection Byproducts Rule. The LT2ESWTR is to address risk from microbial pathogens, specifically *Cryptosporidium*, and the Stage 2 DBPR is to address risk from disinfection byproducts. The Committee recommended that the LT2ESWTR require public water systems (PWSs) to monitor their source water for *Cryptosporidium* using EPA Method 1622 or EPA Method 1623. Additional *Cryptosporidium* treatment requirements for PWSs would be based on the source water *Cryptosporidium* levels. EPA intends to take into account the Committee's advice and recommendations embodied in the Agreement when developing the regulations.

To support *Cryptosporidium* monitoring under the LT2ESWTR, the Committee Agreement recommended that "compliance schedules for the LT2ESWTR...be tied to the availability of sufficient analytical capacity at approved laboratories for all large and medium-size affected systems to initiate *Cryptosporidium* and *E.coli* monitoring..."(65 FR 83015, Dec. 29, 2000) (EPA, 2000). Further, the Agreement recommended that *Cryptosporidium* monitoring by large and medium systems begin within six months following rule promulgation. Given the time necessary for EPA to approve a sufficient number of laboratories to assure adequate capacity for LT2ESWTR monitoring, EPA

would need to begin laboratory evaluation prior to promulgation of the rule in order to accommodate such an implementation schedule.

Another factor that warrants initiation of the Lab QA Program prior to promulgation of the LT2ESWTR is grandfathering of monitoring data. The Agreement recommends that systems with "historical" *Cryptosporidium* data that are equivalent to data that would be collected under the LT2ESWTR be afforded the opportunity to use those "historical" (grandfathered) data in lieu of collecting new data under LT2ESWTR. EPA intends to propose such grandfathering provisions in the LT2ESWTR. If EPA indicates that laboratories meet the criteria in the Lab QA Program described today prior to finalizing the LT2ESWTR, systems could develop monitoring data prior to the LT2ESWTR in anticipation of using it as grandfathered data.

EPA's Office of Ground Water and Drinking Water plans to request from OMB an emergency clearance that would enable expeditious implementation of a voluntary Lab QA Program to support *Cryptosporidium* monitoring under the LT2ESWTR. As such, the Agency could begin to evaluate laboratories that can reliably measure for *Cryptosporidium* using EPA Method 1622 and Method 1623. During the effective period of the emergency clearance, EPA intends to submit to OMB for review and approval a final ICR in order to continue data collection for the Lab QA Program.

As part of today's notice, EPA is inviting comment on the Lab QA Program. Under the Lab QA Program, EPA would evaluate labs on a case-by-case basis through evaluating their capacity and competency to reliably measure for the occurrence of *Cryptosporidium* in surface water using EPA Method 1622 or EPA Method 1623. The intent of this notice is not to propose establishing the Lab QA Program through a rulemaking. Rather, the criteria described in section I.C. are intended to provide guidance to laboratories that are interested in participating in the Lab QA Program.

EPA has not yet proposed rulemaking on use of such "historical" data nor on the methods themselves under the LT2ESWTR. As noted above, EPA intends to propose allowing systems to use equivalent "historical" data in lieu of collecting new data. EPA anticipates the data generated by labs which meet the evaluation criteria would be very high quality, thus increasing the likelihood that such data would warrant consideration as acceptable "grandfathered" data. However, lab evaluation would not guarantee that data generated will be acceptable as "grandfathered" data, nor would failure to meet evaluation criteria necessarily preclude use of "grandfathered" data. For these reasons, EPA is not establishing the Lab QA Program through rulemaking, but rather as a discretionary and voluntary program under the Safe Drinking Water Act, section 1442 (42 USC 300j-1(a)).

A. What is the purpose of the laboratory quality assurance evaluation program?

The purpose of the Lab QA Program is to identify laboratories that can reliably measure for the occurrence of *Cryptosporidium* in surface water. Existing laboratory certification programs do not include *Cryptosporidium* analysis. This program is designed to assess and confirm the capability of laboratories to perform *Cryptosporidium* analyses. The program will assess whether laboratories meet the recommended personnel and laboratory criteria in today's notice. This evaluation program is voluntary for laboratories. In the LT2ESWTR, however, EPA intends to require systems to use

approved (or certified) laboratories when conducting *Cryptosporidium* monitoring under the LT2ESWTR.

B. Why has EPA selected Methods 1622 and 1623 as the basis for determining the data quality of laboratories that measure for Cryptosporidium?

EPA Method 1622 and EPA Method 1623 were developed as improved alternatives to the ICR Protozoan Method (EPA, 1996). EPA validated Method 1622 for the determination of *Cryptosporidium* in ambient water in August 1998 and distributed an interlaboratory validated draft method in January 1999. In addition, EPA validated Method 1623 for the simultaneous determination of *Cryptosporidium* (and *Giardia*) in ambient water in February 1999 and distributed a validated draft method in April 1999.

In April 2001, EPA revised and updated Method 1622 (EPA-821-R-01-026) (EPA, 2001a) and Method 1623 (EPA-821-R-01-025) (EPA, 2001b) based on the following: laboratory feedback, the development of equivalent filters and antibodies for use with the methods, and method performance data generated during the ICR Supplemental Surveys (EPA, 2001e). The results of these studies are documented in the Method 1622 interlaboratory validation study report (EPA-821-R-01-027) (EPA, 2001c) and the Method 1623 interlaboratory validation study report (EPA-821-R-01-028) (EPA, 2001d).

C. What criteria should I use to determine if my laboratory should apply?

A laboratory that is interested in participating in the Lab QA Program currently should be operating in accordance with its QA plan (developed by the laboratory) for *Cryptosporidium* analyses. In addition, an interested laboratory should demonstrate its capacity and competency to analyze *Cryptosporidium* using the following recommended criteria:

1. Recommended personnel criteria:

Principal Analyst/Supervisor (1 per laboratory) should have:

- BS/BA in microbiology or closely related field
- A minimum of 1 year of continuous bench experience with *Cryptosporidium* and immunofluorescent assay (IFA) microscopy
- A minimum of six months experience using EPA Method 1622 and/or EPA Method 1623
- A minimum of 100 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 50 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method (EPA, 1996)) for the specific analytical procedure they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed principal analyst/supervisor

Other Analysts (no minimum number of analysts per laboratory) should have:

- Two years of college (or equivalent) in microbiology or closely related field
- A minimum of six months of continuous bench experience with *Cryptosporidium* and IFA microscopy
- A minimum of three months experience using EPA Method 1622 and/or EPA Method 1623

- A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method) for the specific analytical procedures they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed other analysts

Technician(s) (no minimum number of technicians per laboratory) should have:

- Three months experience with the specific parts of the procedure they will be performing
- A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an analyst approved to conduct analysis for the ICR Protozoan Method) for the specific analytical procedures they will be using
- Submit to EPA, along with the application package, resumes detailing the qualifications of the laboratory's proposed technician(s)

2. Recommended laboratory criteria:

- Appropriate instrumentation as described in EPA Methods 1622 and 1623 (EPA, 2001a,b)
- Equipment and supplies as described in EPA Methods 1622 and 1623 (EPA 2001a, 2001b)
- Detailed laboratory standard operating procedures for each version of the method that the laboratory will use to conduct the *Cryptosporidium* analyses
- Laboratory should provide a current copy of the table of contents of their laboratory's quality assurance plan for protozoa analyses
- EPA Method 1622 or EPA Method 1623 initial demonstration of capability (IDC) data, which include precision and recovery (IPR) test results and matrix spike/matrix spike duplicate (MS/MSD) test results for *Cryptosporidium*. EPA intends to evaluate the IPR and MS/MSD results against the performance acceptance criteria in the April 2001 version of EPA Method 1622 or EPA Method 1623 (EPA, 2001a, 2001b).

D. How can I obtain an application package?

After the OMB clearance described above, EPA plans to make applications available on EPA's website at www.epa.gov/safewater/cryptolabapproval.html. Completed applications should be sent to: EPA's Laboratory Quality Assurance Evaluation Program Coordinator, c/o Dyncorp I&ET, Inc., 6101 Stevenson Avenue, Alexandria, VA 22304-3540. If a laboratory does not have access to the Internet, the laboratory may contact Dyncorp I&ET, Inc. to request an application package.

E. If I demonstrate my laboratory's capacity and competency according to the the personnel and laboratory criteria, what do I do next?

After the laboratory submits to EPA an application package including supporting documentation, EPA intends to conduct the following steps to complete the process:

1. Upon receipt of a complete package, EPA contacts the laboratory for follow-up information and to schedule participation in the performance testing program.
2. EPA sends initial proficiency testing (IPT) samples to the laboratory (unless the laboratory has already successfully analyzed such samples under EPA's Protozoan PE program). IPT samples packets consist of eight spiked samples shipped to the laboratory within a standard matrix.
3. The laboratory analyzes IPT samples and submits data to EPA.

4. EPA conducts an on-site evaluation and data audit.
5. The laboratory analyzes ongoing proficiency testing (OPT) samples three times per year and submits the data to EPA. OPT sample packets consist of three spiked samples shipped to the laboratory within a standard matrix.
6. EPA contacts laboratories by letter within 60 days of their laboratory on-site evaluation to confirm whether the laboratory has demonstrated its capacity and competency for participation in the program.

F. My laboratory has already submitted initial demonstration of capability (IDC) and initial performance testing (IPT) data as part of the EPA Protozoan Performance Evaluation (PE) Program. Do I have to perform this demonstration testing again?

No. If a laboratory currently participates in the EPA Protozoan PE Program and acceptable IDC and IPT data have already been submitted (for the version of the method that the laboratory will use to conduct *Cryptosporidium* analyses), EPA would not expect the laboratory to repeat IDC and IPT analyses.

Section II: Paperwork Reduction Act

The information collection requirements in this notice have been submitted for approval to the OMB under the Paperwork Reduction Act, 44 U.S.C. 3501 et seq. An ICR document has been prepared by EPA (ICR No. 2052.02) and a copy may be obtained from Susan Auby by mail at Collection Strategies Division; EPA (2822); 1200 Pennsylvania Ave., NW, Washington, DC 20460, by email at auby.susan@epamail.epa.gov, or by calling (202) 260-4901. A copy may also be downloaded off the internet at http://www.epa.gov/icr.

Since the EPA would solicit information in application packages, including supporting documentation, analytical data, and other pertinent information from laboratories that are interested in participating in the voluntary Lab QA Program, the Agency is required to submit an ICR to OMB for review and approval. Entities potentially affected by this action include public and private laboratories that wish to be evaluated to determine if they can reliably measure for the occurrence of *Cryptosporidium* in surface waters that are used for drinking water sources using EPA Method 1622 or Method 1623.

The burden estimate for the Lab QA Program information collection includes all the burden hours and costs required for gathering information, and developing and maintaining records associated with the Lab QA Program. The annual public reporting and recordkeeping burden for this collection of information is estimated for a total of 60 respondents and an average 78 hours per response for a total of 4,676 hours at a cost of $123,650. This estimate assumes that laboratories participating in the Lab QA program have the necessary equipment needed to conduct the analyses. Therefore, there are no start-up costs. The estimated total annual capital costs is $0.00. The estimated Operation and Maintenance (O&M) costs is $133,880.

Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

An Agency may not conduct or sponsor, and a person is not required to respond to a

collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations are listed in 40 CFR Part 9 and 48 CFR Chapter 15.

Comments are requested on the Agency's need for this information, the accuracy of the provided burden estimates, and any suggested methods for minimizing respondent burden, including through the use of automated collection techniques. Send comments on the ICR to the Director, Collection Strategies Division; EPA (2822); 1200 Pennsylvania Ave., NW, Washington, DC 20460; and to the Office of Information and Regulatory Affairs, Office of Management and Budget, 725 17th St., N.W., Washington, DC 20503, marked "Attention: Desk Officer for EPA." Include the ICR number in any correspondence. Because OMB is required to make a decision concerning the ICR between 30 and 60 days after March 4, 2002, a comment to OMB is best assured of having its full effect if OMB receives it by April 3, 2002. The final ICR approval notice will respond to any OMB or public comments on the information collection requirements contained in today's notice.

References

EPA. 1996. ICR Microbial Laboratory Manual. Office of Research and Development. EPA/600/R-95/178. April 1996.

EPA. 2000. Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee Agreement in Principle. Federal Register. Vol. 65, pp. 83015-83024. December 29, 2000.

EPA. 2001a. EPA Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. Office of Water. Washington, DC 20460. EPA-821-R-01-026. April 2001.

EPA. 2001b. EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. Office of Water. Washington, DC 20460. EPA-821-R-01-025. April 2001.

EPA. 2001c . Interlaboratory Validation Study Results for *Cryptosporidium* Precision and Recovery for EPA Method 1622. Office of Water. Washington, DC 20460. EPA-821-R-01-027. April 2001.

EPA. 2001d. Interlaboratory Validation Study Results for the Determination of *Cryptosporidium* and *Giardia* Using EPA Method 1623. Office of Water. Washington, DC 20460. EPA-821-R-01-028. April 2001.

EPA. 2001e. Implementation and Results of the Information Collection Rule Supplemental Surveys. Office of Water. Washington, DC 20460. EPA-815-R-01-003. February 2001.

_____Date

APPENDIX B

Federal Register Notice:
Laboratory Quality Assurance Evaluation Program/
Information Collection Request
[Published March 4, 2002]

ENVIRONMENTAL PROTECTION AGENCY
[FRL-]

Agency Information Collection Activities: Submission for OMB Review; Comment Request; EPA Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act/ Laboratory approval for the Long Term 2 Enhanced Surface Water Treatment Rule

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: In compliance with the Paperwork Reduction Act (44 U.S.C. 3501 et seq.), this document announces that the following Information Collection Request (ICR) has been forwarded to the Office of Management and Budget (OMB) for review and approval: EPA Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act, OMB Control Number 2040-0246, expiration date of July 31, 2002. The ICR describes the nature of the information collection and its expected burden and cost; where appropriate, it includes the actual data collection instrument.

DATES: Comments must be submitted on or before [Insert date 30 days after publication in the FEDERAL REGISTER].

ADDRESSES: Send comments, referencing EPA ICR No.2067.02 and OMB Control No.2040-0246, to the following addresses: Susan Auby, U.S. Environmental Protection Agency, Collection Strategies Division (Mail Code 2822), 1200 Pennsylvania Avenue, N.W., Washington, DC 20460; and to Office of Information and Regulatory Affairs, Office of Management and Budget (OMB), Attention: Desk Officer for EPA, 725 17th Street, N.W., Washington, DC 20503.

FOR FURTHER INFORMATION CONTACT: For a copy of the ICR contact Susan Auby at EPA by phone at (202) 260-4901, by E-mail at auby.susan@epamail.epa.gov, or download off the Internet at http://www.epa.gov/icr and refer to EPA ICR No. 2067.02, the ICR number has changed from the last notice. All requests should refer to EPA ICR No. 2067.02 and not EPA ICR No. 2052.02. For technical inquiries, contact Mary Ann Feige, EPA, Office of Ground Water and Drinking Water, Technical Support Center, 26 West Martin Luther King Drive (MS-140), Cincinnati, Ohio 45268, fax number, (513) 569-7191, e- mail address, feige.maryann@epa.gov.

SUPPLEMENTARY INFORMATION:

Title: EPA Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (OMB Control No. 2040-0246 ; EPA ICR No. 2067.01) expiring 7/31/02 . This is a request for extension of a currently approved collection.

Abstract: Section I: Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* Under the Safe Drinking Water Act

In September 2000, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee (Committee) signed an Agreement in Principle (Agreement) (65 FR 83015, Dec. 29, 2000) (EPA, 2000) with consensus recommendations for two future drinking water regulations: The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectants and Disinfection Byproducts Rule. The LT2ESWTR is to address risk from microbial pathogens, specifically *Cryptosporidium*, and the Stage 2 DBPR is to address risk from disinfection byproducts. The Committee recommended that the LT2ESWTR require public water systems (PWSs) to monitor their source water for *Cryptosporidium* using EPA Method 1622 or EPA Method 1623. Additional *Cryptosporidium* treatment requirements for PWSs

would be based on the source water *Cryptosporidium* levels. To support Cryptosporidium monitoring under the LT2ESWTR, the Committee Agreement recommended that ``compliance schedules for the LT2ESWTR * * * be tied to the availability of sufficient analytical capacity at approved laboratories for all large and medium-size affected systems to initiate *Cryptosporidium* and *E.coli* monitoring * * * " (65 FR 83015, Dec. 29, 2000) (EPA, 2000). Further, the Agreement recommended that *Cryptosporidium* monitoring by large and medium systems begin within six months following rule promulgation. Given the time necessary for EPA to approve a sufficient number of laboratories to assure adequate capacity for LT2ESWTR monitoring, EPA would need to begin laboratory evaluation prior to promulgation of the rule in order to accommodate such an implementation schedule. Another factor that warrants initiation of the Lab QA Program prior to promulgation of the LT2ESWTR is grandfathering of monitoring data. The Agreement recommends that systems with ``historical" *Cryptosporidium* data that are equivalent to data that would be collected under the LT2ESWTR be afforded the opportunity to use those ``historical" (grandfathered) data in lieu of collecting new data under LT2ESWTR. EPA intends to propose such grandfathering provisions in the LT2ESWTR. If EPA indicates that laboratories meet the criteria in the Lab QA Program described today prior to finalizing the LT2ESWTR, systems could develop monitoring data prior to the LT2ESWTR in anticipation of using it as grandfathered data. Under the Lab QA Program, EPA would evaluate labs' capacity and competency to reliably measure for the occurrence of *Cryptosporidium* in surface water using EPA Method 1622 or EPA Method 1623. The intent of this notice is not to propose establishing the Lab QA Program through a rulemaking. Rather, the criteria described in section I.C. are intended to provide guidance to laboratories that are interested in participating in the Lab QA Program. EPA anticipates the data generated by labs which meet the evaluation criteria would be very high quality, thus increasing the likelihood that such data would warrant consideration as acceptable ``grandfathered" data. However, lab evaluation would not guarantee that data generated will be acceptable as ``grandfathered" data, nor would failure to meet evaluation criteria necessarily preclude use of ``grandfathered" data. For these reasons, EPA is not establishing the Lab QA Program through rulemaking, but rather as a discretionary and voluntary program under the Safe Drinking Water Act, section 1442 (42 USC 300j-1(a)).

A. What Is the Purpose of the Laboratory Quality Assurance Evaluation Program?

The purpose of the Lab QA Program is to identify laboratories that can reliably measure for the occurrence of Cryptosporidium in surface water. Existing laboratory certification programs do not include Cryptosporidium analysis. This program is designed to assess and confirm the capability of laboratories to perform Cryptosporidium analyses. The program will assess whether laboratories meet the recommended personnel and laboratory criteria in today's notice. This evaluation program is voluntary for laboratories. In the LT2ESWTR, however, EPA intends to require systems to use approved (or certified) laboratories when conducting *Cryptosporidium* monitoring under the LT2ESWTR.

B. How Can I Obtain an Application Package?

After the OMB clearance described above, EPA plans to make applications available on EPA's website at www.epa.gov/safewater/ cryptolabapproval.html. Completed applications should be sent to: EPA's Laboratory Quality Assurance Evaluation Program Coordinator, c/o DynCorp, 6101 Stevenson Avenue, Alexandria, VA 22304-3540. If a laboratory does not have access to the Internet, the laboratory may contact DynCorp to request an application package. Applications may be submitted at any time.

C. If I Demonstrate My Laboratory's Capacity and Competency According to the Personnel and Laboratory Criteria, What Happens Next?

After the laboratory submits to EPA an application package including supporting documentation, EPA intends to conduct the following steps to complete the process:
1) Upon receipt of a complete package, EPA contacts the laboratory for follow-up information and to schedule participation in the performance testing program.
2) EPA sends initial proficiency testing (IPT) samples to the laboratory. IPT samples packets consist of eight spiked samples shipped to the laboratory within a standard matrix.
3) The laboratory analyzes the IPT samples and submits data to EPA. EPA intends to have the laboratory's IPT data meet the IPT criteria of greater than 10% mean recovery and less than 71% relative standard deviation (these criteria were developed based on results from the first six rounds of the EPA PE program). This approach will be used unless unforeseen circumstances merit a reassessment of the approach.
4) EPA conducts an on-site evaluation and data audit. Checklist for evaluation and audit is included in ICR.
5) The laboratory analyzes ongoing proficiency testing (OPT) samples three times per year and submits the data to EPA. OPT sample packets consist of three spiked samples shipped to the laboratory within a standard matrix. The results of the laboratory's OPT data must meet the OPT criteria which will be calculated for each round of OPT testing using only the data from that round. EPA intends to calculate the lower limit as less than 2 standard deviations from the pooled mean using log it transformed data and intends to calculate the maximum RSD as 2 times the pooled RSD. This approach will be used unless unforeseen circumstances merit a reassessment of the approach.
6) EPA contacts laboratories by letter within 60 days of their laboratory on-site evaluation to confirm whether the laboratory has demonstrated its capacity and competency for participation in the program.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations are listed in 40 CFR part 9 and 48 CFR Chapter 15. The Federal Register document required under 5 CFR 1320.8(d), soliciting comments on this collection of information was published on March 4, 2002 (FR). Three comments were received.

Comments requested further information on the details of the Lab Quality Assurance Program. In response, EPA has added supplementary information to the ICR, including the program application, which includes the self-audit checklist detailing the items that will be evaluated during the on-site evaluation. EPA also has also developed a webpage to provide further information on the program. The website can be accessed at http://www.epa.gov/safewater/lt2/cla_final.html.

Commenters expressed concern that the Lab QA Program does not address the Agency's obligation under the FACA Agreement in Principle to identify adequate laboratory capacity to implement LT2ESWTR. The Lab QA Program does assess laboratory capacity through questions on the application on current and potential laboratory capacity to analyze *Cryptosporidium* samples and the on site evaluations. This information will be compiled as laboratory applications are received, and will be updated during on-site evaluations. The on-site evaluation will allow EPA to validate lab capacity reported to EPA.

Comments were received on the burden estimates. Because laboratories that wish to begin using EPA Methods 1622 and 1623 are required by the methods to purchase the equipment necessary to demonstrate initial acceptable performance, and because this is a method requirement, rather than a program requirement (laboratories can perform the methods without ever participating in the program), the burden estimates assume that no capital costs will be incurred by laboratories participating in the program over and above the costs that would be incurred simply to use the method. Because the program application requires the laboratories applying for approval under the program to submit initial performance data, laboratories that meet these requirements should already have the capacity to perform Methods 1622 or 1623 and therefore will not incur start-up costs.

Commenters wanted to know if training would be available for labs needing help. EPA will provide limited training to laboratories needing assistance with the performance of Methods 1622 and 1623. Information on training will be posted on EPA's website as it becomes available.

Commenters wanted to know the earliest date that acceptable grandfathered data could be generated. EPA is aware of the issues regarding grandfathered data acceptability and will address these issues in the proposed LT2ESWTR. These issues are outside of the scope of this ICR.

Burden Statement: The annual public reporting and record keeping burden for this collection of information is estimated to average 18 hours per response. Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information. Respondents/Affected Entities: Testing Laboratories Estimated Number of Respondents: 60. Frequency of Response: 3 times per year. Estimated Total Annual Hour Burden: 4347 hours. Estimated Total Annualized Capital, O&M Cost Burden: $123,380.

Send comments on the Agency's need for this information, the accuracy of the provided burden estimates, and any suggested methods for minimizing respondent burden, including through the use of automated collection techniques to the addresses listed above. Please refer to EPA ICR No. 2067.02 and OMB Control No. 2040-0246 in any correspondence.

APPENDIX C

Federal Register Notice:
Laboratory Quality Assurance Evaluation Program/
Information Collection Request
[Published October 26, 2005]

ENVIRONMENTAL PROTECTION AGENCY
[OW–2002–0011, FRL–7988–7]

Agency Information Collection Activities; Submission to OMB for Review and Approval; Comment Request; **Laboratory Quality Assurance Evaluation Program for Analysis of Cryptosporidium Under the Safe Drinking Water Act, EPA ICR Number 2067.03, OMB Control Number 2040–0246**

AGENCY: Environmental Protection Agency.
ACTION: Notice.
SUMMARY: In compliance with the Paperwork Reduction Act (44 U.S.C. 3501 et seq.), this document announces that an Information Collection Request (ICR) has been forwarded to the Office of Management and Budget (OMB) for review and approval. This is a request to renew an existing approved collection. This ICR is scheduled to expire on October 31, 2005. Under OMB regulations, the Agency may continue to conduct or sponsor the collection of information while this submission is pending at OMB. This ICR describes the nature of the information collection and its estimated burden and cost.
DATES: Additional comments may be submitted on or before November 25, 2005.

ADDRESSES: Submit your comments, referencing docket ID number OW– 2002–0012, to (1) EPA online using EDOCKET (our preferred method), by email to ow-docket@epamail.epa.gov, or by mail to: EPA Docket Center, Environmental Protection Agency, Water Docket, Mail Code 4101T, 1200 Pennsylvania Ave., NW., Washington, DC 20460.
FOR FURTHER INFORMATION CONTACT: Sean Conley, Office of Groundwater and Drinking Water, (Mail Code 4607M), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460; telephone number: 202–564– 1781; fax number: 202–564–3767; e-mail address: conley.sean@epa.gov. For technical inquiries, contact Carrie Moulton, EPA, Office of Ground Water and Drinking Water, Technical Support Center, 26 West Martin Luther King Drive (MS–140), Cincinnati, Ohio 45268; fax number: (513) 569–7191; email address: moulton.carrie@epa.gov.
SUPPLEMENTARY INFORMATION: EPA has submitted the following ICR to OMB for review and approval according to the procedures prescribed in 5 CFR 1320.12. On June 3, 2005 (70 FR 32607), EPA sought comments on this ICR pursuant to 5 CFR 1320.8(d). EPA has addressed the comments received.

EPA has established a public docket for this ICR under Docket ID No. OW– 2002–0012, which is available for public viewing at the Water Docket in the EPA Docket Center (EPA/DC), EPA West, Room B102, 1301 Constitution Ave.,
NW., Washington, DC. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Reading Room is (202) 566–1744, and the telephone number for the Water Docket is (202) 566–2426. An electronic version of the public docket is available through EPA Dockets (EDOCKET) at http://www.epa.gov/edocket. Use EDOCKET to submit or view public comments, access the index listing of the contents of the public docket, and to access those documents in the public docket that are available electronically. Once in the system, select ‘‘search,’’ then key in the docket ID number identified above.

Any comments related to this ICR should be submitted to EPA and OMB within 30 days of this notice. EPA’s policy is that public comments, whether submitted electronically or in paper, will be made available for public viewing in EDOCKET as EPA receives them and without change, unless the comment contains copyrighted material, CBI, or other information whose public disclosure is restricted by statute. When EPA identifies a comment containing

copyrighted material, EPA will provide a reference to that material in the version of the comment that is placed in EDOCKET. The entire printed comment, including the copyrighted material, will be available in the public docket. Although identified as an item in the official docket, information claimed as CBI, or whose disclosure is otherwise restricted by statute, is not included in the official public docket, and will not be available for public viewing in EDOCKET. For further information about the electronic docket, see EPA's Federal Register notice describing the electronic docket at 67 FR 38102 (May 31, 2002), or go to http://www.epa.gov/edocket.

Title: Laboratory Quality Assurance Evaluation Program for Analysis of Cryptosporidium under the Safe Drinking Water Act.

Abstract: Under the Laboratory Quality Assurance Evaluation Program, EPA evaluates labs on a case-by-case basis through evaluating their capacity and competency to reliably measure for the occurrence of Cryptosporidium in surface water using EPA Method 1622 or EPA Method 1623. To obtain approval under the program, the laboratory must submit an application package and provide: a demonstration of availability of qualified personnel and appropriate instrumentation, equipment and supplies; a detailed laboratory standard operating procedure for each version of the method that the laboratory will use to conduct the Cryptosporidium analyses; a current copy of the table of contents of their laboratory's quality assurance plan for protozoa analyses; and an initial demonstration of capability data for EPA Method 1622 or EPA Method 1623, which include precision and recovery test results and matrix spike/matrix spike duplicate test results for Cryptosporidium. After the laboratory submits to EPA an application package including supporting documentation, EPA and the laboratory conduct the following steps to complete the process:

1. EPA contacts the laboratory for follow-up information and to schedule participation in the performance testing program.

2. EPA sends initial proficiency testing samples to the laboratory (unless the laboratory has already successfully analyzed such samples under EPA's Protozoan PE program). These sample packets consist of eight spiked samples shipped to the laboratory within a standard matrix.

3. The laboratory analyzes initial proficiency testing samples and submits data to EPA.

4. EPA conducts an on-site evaluation and data audit.

5. The laboratory analyzes ongoing proficiency testing samples three times per year and submits the data to EPA. These sample packets consist of three spiked samples shipped to the laboratory within a standard matrix.

6. EPA contacts laboratories by letter within 60 days of their laboratory onsite evaluation to confirm whether the laboratory has demonstrated its capacity and competency for participation in the program.

The procedure for obtaining an application package, the criteria for demonstrating capacity and competency, and other guidance to laboratories that are interested in participating in the program, are provided at http://www.epa.gov/ safewater/lt2/cla_final.html.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations in 40 CFR are listed in 40 CFR part 9 and are identified on the form and/or instrument, if applicable.

Burden Statement: The annual public reporting and recordkeeping burden for this collection of information is estimated to average 19 hours per response. Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and

disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

Respondents/Affected Entities: Public and private water testing laboratories.

Estimated Number of Respondents: 22.

Frequency of Response: Three times per year.

Estimated Total Annual Hour Burden: 3,980.

Estimated Total Annual Cost: $275,000, includes $109,000 annualized capital or O&M costs.

Changes in the Estimates: There is a decrease of 367 hours in the total estimated burden currently identified in the OMB Inventory of Approved ICR Burdens. This decrease is just an adjustment to the estimate.

Dated: October 18, 2005.

Sara Hisel-McCoy,

Acting Director, Collection Strategies Division.

[FR Doc. 05–21370 Filed 10–25–05; 8:45 am] BILLING CODE 6560–50–P

APPENDIX D

Federal Register Notice:
Laboratory Quality Assurance Evaluation Program/
Information Collection Request
[Published February 25, 2009]

ENVIRONMENTAL PROTECTION AGENCY

[EPA-HQ-OW-2002-0011; FRL-8776-6]

Agency Information Collection Activities; Proposed Collection; Comment Request; Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* Under the Safe Drinking Water Act (Renewal); EPA ICR No. 2067.04, OMB Control No. 2040-0246

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: In compliance with the Paperwork Reduction Act (PRA) (44 U.S.C. 3501 et seq.), this document announces that EPA is planning to submit a request to renew an existing approved Information Collection Request (ICR) to the Office of Management and Budget (OMB). This ICR is scheduled to expire on May 31, 2009. This notice describes the current ``Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act," hereafter referred to as the ``Lab QA Program," and requests comment on both the program and the renewed paperwork requirements.

DATES: Comments must be submitted on or before April 27, 2009.

ADDRESSES: Submit your comments, identified by Docket ID No. EPA-HQ-OW- 2002-0011, by one of the following methods:

http://www.regulations.gov: Follow the on-line instructions for submitting comments.

Mail: Water Docket, Environmental Protection Agency, Mailcode: 2822T, 1200 Pennsylvania Ave., NW., Washington, DC 20460.

Hand Delivery: Monday through Friday, excluding legal holidays. The telephone number for the Reading Room is (202) 566-1744, and the telephone number for the Water Docket is (202) 566-2426. Such deliveries are only accepted during the Docket's normal hours of operation, and special arrangements should be made for deliveries of boxed information.

Instructions: Direct your comments to Docket ID No. EPA-HQ-OW-2002- 0011. EPA's policy is that all comments received will be included in the public docket without change and may be made available online at http://www.regulations.gov, including any personal information provided, unless the comment includes information claimed to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. Do not submit information that you consider to be CBI or otherwise protected using http://www.regulations.gov or e-mail. Please contact EPA prior to submitting CBI. The http://www.regulations.gov Web site is an ``anonymous access" system, which means EPA will not know your identity or contact information unless you provide it in the body of your comment. If you send an e-mail comment directly to EPA without going through http://www.regulations.gov your e-mail address will be automatically captured and included as part of the comment that is placed in the public docket and made available on the Internet. If you submit an electronic comment, EPA recommends that you include your name and other contact information in the body of your comment and with any disk or CD-ROM you submit. If EPA cannot read your comment due to technical difficulties and

cannot contact you for clarification, EPA may not be able to consider your comment. Electronic files should avoid the use of special characters, any form of encryption, and be free of any defects or viruses. For additional information about EPA's public docket visit the EPA Docket Center homepage at http://www.epa.gov/epahome/dockets.htm.

FOR FURTHER INFORMATION CONTACT: Carrie Miller, EPA, Office of Ground Water and Drinking Water, Technical Support Center, 26 West Martin Luther King Drive (MS-140), Cincinnati, Ohio 45268; e-mail address: miller.carrie@epa.gov.

SUPPLEMENTARY INFORMATION:

How Can I Access the Docket and/or Submit Comments?

EPA has established a public docket for this ICR under Docket ID No. EPA-HQ-OW-2002-0011, which is available for online viewing at http://www.regulations.gov, or in person viewing at the Water Docket in the EPA Docket Center (EPA/DC), EPA West, Room 3334, 1301 Constitution Ave., NW., Washington, DC. The EPA/DC Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Reading Room is 202-566-1744, and the telephone number for the Water Docket is 202-566-2426.

Use http://www.regulations.gov to obtain a copy of the draft collection of information, submit or view public comments, access the index listing of the contents of the docket, and to access those documents in the public docket that are available electronically. Once in the system, select ``search,'' then key in the docket ID number identified in this document.

What Information Is EPA Particularly Interested in?

Pursuant to section 3506(c)(2)(A) of the PRA, EPA specifically solicits comments and information to enable it to:

(i) Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the Agency, including whether the information will have practical utility;

(ii) Evaluate the accuracy of the Agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;

(iii) Enhance the quality, utility, and clarity of the information to be collected; and

(iv) Minimize the burden of the collection of information on those who are to respond, including through the use of appropriate automated electronic, mechanical, or other technological collection techniques or other forms of information technology, e.g., permitting electronic submission of responses. In particular, EPA is requesting comments from very small businesses (those that employ less than 25) on examples of specific additional efforts that EPA could make to reduce the paperwork burden for very small businesses affected by this collection.

EPA is also interested in any other comments regarding the improvements to the Lab QA Program described in this notice.

What Should I Consider When I Prepare My Comments for EPA?

You may find the following suggestions helpful for preparing your comments:

1. Explain your views as clearly as possible and provide specific examples.

2. Describe any assumptions that you used.

3. Provide copies of any technical information and/or data you used that support your views.

4. If you estimate potential burden or costs, explain how you arrived at the estimate that you provide.

5. Offer alternative ways to improve the collection activity.

6. Make sure to submit your comments by the deadline identified under DATES.

7. To ensure proper receipt by EPA, be sure to identify the docket ID number assigned to this action in the subject line on the first page of your response. You may also provide the name, date, and Federal Register citation.

What Information Collection Activity or ICR Does This Apply to?

Affected entities: Entities potentially affected by this action are public and private water testing laboratories. EPA estimates that a total of 65 laboratories will seek to attain or maintain EPA recognition under the Lab QA Program. This estimate includes 63 laboratories seeking continued recognition under the Lab QA Program and 2 laboratories seeking initial recognition.

Title: Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Renewal).

ICR numbers: EPA ICR No. 2067.04, OMB Control No. 2040-0246.

ICR status: This ICR is currently scheduled to expire on May 31, 2009. An Agency may not conduct or sponsor, and a person is not required to respond to, a collection of information, unless it displays a currently valid OMB control number. Approved OMB control numbers for EPA's regulations in title 40 of the CFR are listed in 40 CFR part 9 of the Federal Register and displayed either by publication of the Federal Register or by other appropriate means, such as on the applicable collection instrument or form.

Abstract: In September 2000, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee (Committee) signed an Agreement in Principle (Agreement) (65 FR 83015, December 29, 2000) (EPA, 2000) with consensus recommendations for two future drinking water regulations: the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectants and Disinfection Byproducts Rule. The LT2ESWTR was to address risk from microbial pathogens, specifically *Cryptosporidium*. The Committee recommended that the LT2ESWTR require public water systems (PWSs) to monitor their source water for *Cryptosporidium* using EPA Method 1622 or EPA Method 1623. Additional *Cryptosporidium* treatment requirements for PWSs would be based on the source water *Cryptosporidium* levels. EPA took into account the Committee's advice and recommendations as it developed the LT2ESWTR, which was published on January 5, 2006.

Under the LT2ESWTR, EPA requires public water systems to use approved laboratories when conducting *Cryptosporidium* monitoring. In the preamble to the LT2ESWTR as well as several other notices, EPA has described the criteria for approval of laboratories to analyze *Cryptosporidium* samples under the LT2ESWTR. See 71 FR 727 (January 5, 2006) and 67 FR 9731 (March 4, 2002). The Lab QA Program, as revised, is described in this notice. The purpose of the Lab QA Program is to identify laboratories that can reliably measure for the occurrence of *Cryptosporidium* in surface water and to ensure that approved laboratories maintain that

capability. Other, State-based laboratory oversight programs do not currently address approval of laboratories for the *Cryptosporidium* analysis required by the LT2ESWTR.

Through today's notice, EPA is inviting comment on refinements to the information collected to support EPA's Lab QA Program. As of May 2007, EPA concluded that sufficient laboratory capacity exists for the LT2ESWTR. As a result, EPA has generally postponed evaluation of additional laboratories, including commercial, county, municipal and utility laboratories, until further notice. Subject to the availability of resources, EPA will consider evaluation of State and EPA Regional laboratories on a case-by-case basis, based on the role that States and EPA Regions play in the certification and approval programs for laboratories. The Lab QA Program is continuously being refined and updated as new information and technologies become available. The program will continue to evolve and EPA will continue to revise and update burden estimates, as needed, with any subsequent ICR.

Approved laboratories will have demonstrated, and are to continue to demonstrate, proficient and reliable detection and enumeration of *Cryptosporidium* in surface water sources for public water systems. They will have passed all elements in the Lab QA Program and continue to successfully participate in all program activities. Approved laboratories are responsible for notifying EPA of losses of key personnel or essential equipment and changes in policies or procedures that directly affect the validity of data or any other change affecting the capability of the laboratory including change in location. Participating laboratories are to also demonstrate ongoing capability and method performance by following all applicable method quality control (QC) procedures, analyzing ongoing proficiency testing (PT) samples (generally three times per year), submitting requested data to EPA, and participating in periodic re-evaluations.

The Lab QA Program procedures have been updated to reflect that the minimum recovery for *Cryptosporidium* in ongoing precision and recovery (OPR) samples is now 22 percent, updated from the original 11 percent. This updated minimum recovery is based on an updated data set and should provide a better assessment of laboratory performance than the original value for the following reasons: (1) The data set is more current and is based on more samples (a total of 333); (2) 52 more laboratories are included in the data set; (3) data were generated using the 2005 version of Method 1623, which is the required version for LT2ESWTR analyses; (4) data were generated using filters currently used to analyze LT2ESWTR samples rather than those filters used originally; and (5) the number of oocysts spiked into the samples was unknown to the laboratories. Calculations for the updated criteria are available in Docket ID No. EPA-HQ-OW-2002-0011. Laboratories are to now document a minimum of 22 percent recovery for OPR samples in an updated QC chart prior to analysis of LT2ESWTR samples at the frequency required in section 9.7 of the method.

The ongoing PT sample packets generally consist of three spiked samples shipped to the laboratory within a standard matrix. If a laboratory submits poor PT results, 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 QC data, and investigation of problems with reagents and equipment. Repeated failure to demonstrate laboratory capability and acceptable method performance may result in suspension or downgrading of approval status as outlined later in this section.

EPA may re-evaluate laboratories participating in the program to verify *Cryptosporidium* laboratory quality assurance (QA) on both an ``as-needed'' and periodic basis (generally not exceeding once every three years). In the case of a periodic assessment, EPA will generally

notify the laboratory that they are due for re-evaluation and request a package with documentation of personnel status, equipment maintenance, standard operating procedures, training records, and QC charts. After the package has been received, it will be evaluated for completeness. EPA generally contacts the laboratory within 15 days of package submission if information is missing. When a complete package has been received, the following steps will complete the process:

1. The laboratory will send positive staining control and OPR slides for evaluation by EPA.

2. The laboratory will order blind slides spiked with *Cryptosporidium* from a qualified vendor for each analyst. Each analyst will perform an independent count of one slide. The results and slides will be submitted to a technical auditor.

3. EPA will schedule an on-line Internet analyst verification of performance for microscopists to demonstrate their ability to identify *Cryptosporidium* oocysts.

4. EPA conducts a one-day on-site evaluation that will primarily focus on method performance and data recording. Laboratory personnel will be asked to order blind oocyst suspensions for use in sample and IMS control spiking in the presence of an auditor, and then complete the analyses within applicable method holding times and send results to EPA.

5. EPA will send the laboratory a report detailing all findings, generally within 60 days after the evaluation is complete. The laboratory is then asked to provide written responses to any deficiencies identified in the report within 60 days. Provided all responses to the deficiencies cited in the report are acceptable, the Lab QA Program will then base its decision for continued laboratory approval on PT results, quality of the positive control and OPR slide, slide counts, Internet analyst verification, on-site evaluation and recovery values for blind analyses initiated during the on-site evaluation.

State and EPA Regional Laboratories may contact the laboratory approval manager regarding new application submissions. Subject to available resources, EPA estimates that up to two State or EPA Regional Laboratories will seek first-time approval each year. Laboratories seeking approval under the program must submit an application package and provide: a demonstration of availability of qualified personnel and appropriate instrumentation, equipment and supplies; detailed laboratory standard operating procedures; a current copy of the table of contents of their laboratory's QA plan for protozoa analyses; and an initial demonstration of capability data for EPA Method 1623, which includes initial precision and recovery IPR test results and matrix spike/matrix spike duplicate (MS/MSD) test results for *Cryptosporidium*. After EPA completes its review of the application, the Agency will contact the laboratory for follow-up information and to schedule shipment of initial PT samples consisting of eight spiked samples within a standard matrix. EPA then generally conducts an on-site evaluation and data audit. Further information is provided at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html. The Agency notes that completion of an application by a laboratory does not ensure that the Agency will act on the laboratory's request; interested laboratories are encouraged to contact the laboratory approval manager prior to investing substantial effort towards their application. Further, a decision by the Agency to review an application, to send initial PT samples, and/or to schedule or conduct an on-site evaluation and data evaluation, does not ensure that the review process will be completed or that the laboratory will ultimately be approved. Decisions will be made based on the facts associated with a particular application and actions will be taken as Agency resources permit.

Approved laboratories that do not continue to meet the criteria for the Lab QA Program may have their status downgraded to provisional or have their approval suspended. Details of the basis for downgrading or suspending a laboratory's approval are provided in the section entitled ``Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* in Water Under the Long Term 2 Enhanced Surface Water Treatment Rule'' (see the following section). Provided EPA has sufficient resources to review requests for upgrade or reinstatement, laboratories may have to undertake additional activities such as analyzing additional PT samples, undergoing an on-site evaluation, and/or counting blind spiked slides in order to have their status upgraded or their approval reinstated. Details regarding additional activities that may be required are provided in the next section.

Clarification of Basis and Procedures for Downgrading/Suspending Approval of Laboratories for the Analysis of *Cryptosporidium* in Water Under the Long Term 2 Enhanced Surface Water Treatment Rule

EPA's Office of Ground Water and Drinking Water, in the Office of Water, has developed a detailed description of the procedures and criteria used in actions concerning approving, downgrading and suspending laboratories for analysis of drinking water contaminants.

In order to assume primary enforcement responsibility for the drinking water regulations, a State must either have available laboratory facilities, approved by the Administrator, capable of conducting analytical measurements of drinking water contaminants, or establish and maintain its own program for approval of laboratories. States wishing to adapt these procedures and criteria for their own approval program should revise it to accurately reflect their State approval program.

This section is intended to clarify EPA's intended practices and procedures for laboratory approval, downgrading or suspension for analysis of *Cryptosporidium* under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and to reflect good laboratory practice and standard proficiency evaluation in the industry; it is not a regulation. While EPA intends to generally follow the procedures laid out in this section, not every situation is reflected in these procedures and EPA may need to address case-specific situations in ways that differ from the procedures spelled out here. EPA welcomes comment on these procedures and may decide to revise them at any time in the future to reflect changes to its approach or to clarify and update the text.

``Approved Laboratories'' have demonstrated, and continue to demonstrate, proficient and reliable detection and enumeration of *Cryptosporidium* in surface water sources for public water systems. They have passed all elements in the Lab QA Program and continue to successfully participate in all program activities. Approved Laboratories notify the Approval Authority (EPA individual(s) administering the program or State individual(s) administering an equivalent laboratory certification program) of loss of key personnel or essential equipment, change in policies or procedures that directly affect the validity of data, and any other change affecting the capability of the laboratory including change in location.

``Provisionally Approved Laboratories'' have deficiencies but demonstrate their ability to consistently produce data of known quality. They continue to successfully participate in all Lab QA Program activities. A Provisionally Approved Laboratory may analyze drinking water

samples for LT2ESWTR compliance purposes if the laboratory has identified themselves as provisionally approved to their clients and any reports clearly state that the laboratory's status is ``provisionally approved."

``Not Approved" designates a laboratory that has either not participated in the Lab QA Program, or has applied to the program but possesses deficiencies and, in the opinion of the Approval Authority, does not consistently produce data that has met all applicable method QC requirements or has falsified data.

Basis for Downgrading to ``Provisionally Approved" Status

An Approved Laboratory (referred to as ``laboratory") may be downgraded to ``Provisionally Approved" status for *Cryptosporidium* for any of the following reasons:

Failure to analyze samples for the LT2ESWTR according to the December 2005 version of EPA Method 1623 or EPA Method 1622, including all QA/QC criteria;

Failure to document a minimum of 22 percent for on-going precision and recovery values in an updated QC chart prior to analysis of LT2ESWTR samples at the frequency required in section 9.7 of the method;

Failure to demonstrate proficiency based upon acceptable matrix spike recoveries for all modifications of the method procedures per Section 9.1.2 of the method;

Failure to submit valid Proficiency Test (PT) results or meet PT acceptance limits described by the Approval Authority for the first two initial testing events or two out of three regular testing events administered by a vendor authorized by the Approval Authority. The acceptance limits are laboratory mean recovery between 2 standard deviations (SD) of the mean recovery for all approved laboratories in a given test event. Recoveries below the mean recovery minus 2 SD will fail the PT test event. Recoveries higher than the mean recovery plus 2 SD trigger additional evaluation, which may include one or more of the following: (1) On-site evaluation; (2) presence of a proctor when processing PT samples during the next test event; and/or (3) submission of PT microscope slides to the Approval Authority before the expiration of holding time during the next test event;

Failure to submit PT slides within three weeks of PT test event when requested by the Approval Authority;

Failure to maintain records of method modifications per section 9.1.2.2 of the method;

Failure to notify the Approval Authority of loss of key personnel or essential equipment, change in policies or procedures that directly affect the validity of data, or other changes affecting the capability of the laboratory including change in location. Laboratory Approval does not automatically survive such changes; the Approval Authority may request an on-site or off-site evaluation and/or further proof of compliance with all applicable method requirements;

Failure to submit on-site evaluation materials and any other requested information within the time period requested by the Approval Authority; or

Failure to participate satisfactorily in the Approval Authority Lab QA Program and demonstrate proficiency based upon: Sample and method holding time records; analyst verification skills; relative quality of positive staining control and on-going precision recovery (OPR) slides; acceptable performance of QC checks, including but not limited to blind slide counts; and acceptable precision and recovery values for all method variations.

Procedures for Downgrading to ``Provisionally Approved" Status

The Approval Authority will notify the laboratory director or owner of its intent to downgrade after becoming aware of the situation warranting downgrading;

The laboratory director should review the problems cited, and within 30 days of receipt of the letter, send a letter to the Approval Authority specifying immediate corrective actions that are being taken;

The Approval Authority will consider the adequacy of the response and notify the laboratory in writing of its approval status, generally within 14 days of receipt of the laboratory's response;

After the Approval Authority notifies a laboratory, the Approval Authority will post status on the Web site list of laboratories and may schedule an on-site evaluation of the laboratory;

The laboratory should identify and correct its problem(s) to the Approval Authority's satisfaction within 30 days of being notified of the downgrade or have approval status suspended;

A Provisionally Approved laboratory may continue to analyze samples for compliance purposes, but must identify its status as Provisionally Approved on any report;

A laboratory may request that the Approval Authority or State provide technical assistance to help identify and resolve any problem; however, adequate performance is the laboratory's responsibility and Approval Authority assistance should not delay the downgrading procedure.

Basis for Suspending Approval Status

A laboratory may be downgraded from Approved or Provisionally Approved status to ``Not Approved'' for any of the following reasons:

Repeated verification that all applicable method QC requirements have been followed, when in fact they have not all been met;

Repeated failure to document acceptable OPR values prior to analysis of LT2ESWTR samples;

Reporting PT data from another laboratory as its own;

Falsification of data or other deceptive practices including false verification that data submitted to the Data Collection and Tracking System (DCTS) was generated using approved methods and met all method QA/QC criteria;

Refusal to participate in on-site or off-site evaluations conducted by the Approval Authority.

Basis for Suspending Provisionally Approved Status

Failure to provide a letter to the Approval Authority within 30 days that adequately explains what immediate corrective actions were taken;

Failure to identify and correct problems in response to downgrade within 30 days;

Failure to provide accurate OPR control charts to the Approval Authority;

Failure to submit valid PT results for the next two consecutive authorized PT test events within the acceptance limits specified;

Continued failure to use the analytical methodology specified in the regulations;

Failure to correct deviations identified during an on-site evaluation within 30 days; or

Failure to provide requested demonstration, materials and documentation within 30 days, including: acceptable matrix spike recoveries for all method variations per section 9.1.2 of the method; bench sheets, examination forms or OPR charts for any samples requested; remote analyst verification; recent positive staining control and OPR microscope slides, one of each; and blind slide counts for each analyst.

Procedures for Suspension

The Approval Authority will notify the laboratory, in writing, of its intent to suspend approval. If the laboratory wishes to request reconsideration of this decision, it should submit such a request in writing to the Approval Authority within 30 days of receipt of the notice of intent to suspend approval. The laboratory will generally be downgraded immediately to ``provisional approval'' in the interim while the suspension is being considered. If no request for reconsideration is filed, approval will be suspended.

The request for reconsideration should be supported with an explanation of the reasons for the challenge and should be signed by a responsible official from the laboratory such as the president/owner for a commercial laboratory, the laboratory supervisor of a municipal laboratory, or the laboratory director for a State or Regional laboratory.

The Approval Authority will make a decision and notify the laboratory in writing, generally within 30 days of receipt of the request for reconsideration. If the request is determined to be valid, the Approval Authority will take appropriate measures to reevaluate the facility and notify the laboratory, in writing, of its decision, generally within 60 days of the reevaluation.

Denial of the request will generally result in suspension of the laboratory's approval. Once approval is suspended, a public water system may not use the laboratory to analyze source water samples for compliance with LT2ESWTR source water monitoring requirements. The laboratory should notify its clients that it is no longer approved and will not accept any more LT2ESWTR samples for analysis.

Upgrading or Reinstatement of Approval

Subject to the availability of resources, the Approval Authority will consider written requests from the laboratory to seek upgrading or reinstatement of approval. Requests should state the reasons why the laboratory should regain its approval status. The laboratory should demonstrate that all deficiencies have been corrected and successfully complete two consecutive authorized PT test events within acceptance limits for Provisionally Approved laboratories or three consecutive authorized PT test events within acceptance limits for suspended laboratories. The authorized PT test events being described here are those submitted to all laboratories in the Lab QA Program, not special issue blind samples purchased independently from the vendor. The laboratory should provide evidence why the reasons for downgrading or suspension are no longer applicable and explain its technical competence. Acceptable demonstration of technical competence may include an on-site evaluation and/or any other measure the Approval Authority deems appropriate. The Approval Authority will consider compliance history, corrective actions

implemented by the laboratory, effectiveness of corrective actions, and professional judgment of the Approval Authority.

Grievances

Laboratories with grievances during the authorized PT events or regarding participation in the Lab QA Program should immediately contact the Program Manager at the Approving Authority and try to remedy the problem. When the laboratory feels they have not gotten immediate or satisfactory results, they should contact the supervisor at the Approving Authority. The management at the Approving Authority will work with the Program Manager to quickly address grievances. A final decision for all grievances will be made generally within 30 days of contacting the Approving Authority.

Request for Comment

The EPA is soliciting comments on this notice to:

1. Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the Agency, including whether the information will have practical utility;

2. Evaluate the accuracy of the Agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;

3. Enhance the quality, utility, and clarity of the information to be collected;

4. Minimize the burden of the collection of information on those who are to respond, including through use of appropriate automated electronic, mechanical, or other technological collection techniques or other forms of information technology, e.g., permitting electronic submission of responses; and

5. Consider any necessary changes to the Lab QA Program. As an example, EPA is particularly interested in comments from States regarding the potential for their laboratory programs to assume any/all responsibility for the approval and oversight of LT2ESWTR laboratories, including comments on the appropriate timeframes for such. The Agency also welcomes comments regarding the appropriateness of turning to commercial PT providers as the source of PT samples for laboratories, in lieu of the PT program currently administered by the Agency.

Burden Statement: The burden estimate for the Lab QA Program information collection includes all the burden hours and costs required for gathering information, and developing and maintaining records associated with the Lab QA Program. An estimated 65respondents will participate in an average of 4.4 responses per year to include: analysis and reporting of PT samples three times per year, application for initial or re-audit once every three years, off-site re-evaluation activities once every three years, and on-site evaluation once every three years. A small subset of laboratories will perform follow-up activities based on inadequate QA/QC, failed OPRs, incomplete records, delayed communication to EPA or poor PT results. A few laboratories perform more than one method version and will analyze an additional set of PTs amples three times per year. The total annual public reporting and recordkeeping burden for this collection of information is estimated to be 4843 hours at a cost of $269,800.40. The average hours and cost per response for the average of 4.4 responses per year are 16.9 hours and $943.36, respectively. These estimates assume that laboratories participating in the Lab QA

Program have the necessary equipment needed to conduct the analyses. Therefore, there are no start-up costs. The estimated total annual capital cost is $0.00. The total estimated Operation and Maintenance (O&M) costs is $141,929.00.

The ICR provides a detailed explanation of the Agency's estimate, which is only briefly summarized here:

Estimated Total Number of Potential Respondents: 65.

Frequency of Response: Annual.

Estimated Total Average Number of Responses for Each Respondent: 4.4.

Estimated Total Annual Burden Hours: 4843 hours.

Estimated Total Annual Costs: $411,729.40. This includes an estimated burden cost of $269,800.40 and an estimated cost of $141,929.00 for capital investment or maintenance and operational costs.

Are There Changes in the Estimates From the Last Approval?

Changes in burden have occurred due to inflation, re-evaluation of hours for tasks, and improved demonstration of capability. Inflation has increased all operation and maintenance and labor costs accordingly. The increase in the respondent universe has increased the overall burden costs for the respondents. EPA's original estimates for hours to participate and maintain the Lab QA Program were made before the program began. Because the program has matured and several years of QC data have been collected, the burden has changed for performing improved and refined procedures. The burden for some tasks has been estimated and will be re-evaluated as the program progresses. EPA has added the preceding section entitled ``Clarification of Basis and
Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* in Water Under the Long Term 2 Enhanced Surface Water Treatment Rule." Some approved laboratories may have to undertake additional activities to demonstrate continued acceptable performance to EPA, which may increase the burden of participation in the Lab QA Program for those laboratories. EPA estimates that nine laboratories per year may have to undertake additional activities to demonstrate acceptable performance to EPA. These estimates will be corrected as the program continues.

What is the Next Step in the Process for This ICR?

EPA will consider the comments received and amend the ICR as appropriate. The final ICR package will then be submitted to OMB for review and approval pursuant to 5 CFR 1320.12. At that time, EPA will issue another Federal Register notice pursuant to 5 CFR 1320.5(a)(1)(iv) to announce the submission of the ICR to OMB and the opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under FOR FURTHER INFORMATION CONTACT.

Dated: February 19, 2009.
Cynthia C. Dougherty,
Director, Office of Ground Water and Drinking Water.
[FR Doc. E9-4009 Filed 2-24-09; 8:45 am]

BILLING CODE 6560-50-P

APPENDIX E

Comments on February 25, 2009 Federal Register Notice:
Laboratory Quality Assurance Evaluation Program/
Information Collection Request

Docket: EPA-HQ-OW-2002-0011
Agency Information Collection Activities; Submission to OMB for Review and Approval; Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act

Comment On: EPA-HQ-OW-2002-0011-0033
Agency Information Collection Activities; Proposed Collection; Comment Request; Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* Under the Safe Drinking Water Act (Renewal); EPA ICR No. 2067.04, OMB Control No. 2040-0246

Document: EPA-HQ-OW-2002-0011-0037
Comment submitted by John T. Gordy, Senior Environmental Scientist, Water Quality Laboratory, City of Tampa, Florida (FL)

Response: The February 25, 2009 Information Collection Request requested comment specifically on the Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act. The Agency did not solicit comments on EPA Methods 1622/1623 and will, therefore, not be addressing such comments as part of the ICR renewal action. These comments are nonetheless appreciated and will be considered if the Agency entertains changes to the method at a future date.

Document: EPA-HQ-OW-2002-0011-0038
Comment submitted by Dr. L. Manja R. Blazer, Senior Manager, Government Affairs & Market Development, IDEXX Laboratories

Response: EPA appreciates the comments supporting the Agency's program and the efforts to improve the program.

Document: EPA-HQ-OW-2002-0011-0039
Comment submitted by Darrell Osterhoudt, Association of State Drinking Water Administrators (ASDWA)

Response: Thank you very much for your comment and perspective on the program. EPA has added this to the information we will consider regarding State equivalent approval and certification programs.

APPENDIX F

State and EPA Regional Laboratory Participation Process

LT2ESWTR Lab QA State and EPA Regional Participation Process

The purpose of evaluating applications for the Laboratory Quality Assurance (Lab QA) Program is to identify State and EPA Regional laboratories that can reliably measure the occurrence of *Cryptosporidium* in surface water using EPA Method 1622 and/or EPA Method 1623. While this program is voluntary, participating laboratories must: 1) have the equipment required in EPA Method 1622 and/or EPA Method 1623; 2) have experienced personnel; and 3) successfully complete an initial demonstration of capability. The laboratory, personnel, and demonstration criteria are specified in the application cover letter.

EPA will consider evaluation of the State and EPA Regional laboratories on a case-by-case basis, resources permitting. All interested State and EPA Regional laboratories may contact the laboratory approval manager regarding application submission.

Steps for State and EPA Regional Laboratories to participate:

- Step 1. Contact Lab Approval Manager Carrie Miller, Manager, *Cryptosporidium* Laboratory Approval Program U.S. Environmental Protection Agency Office of Ground Water and Drinking Water, Technical Support Center 26 West Martin Luther King Dr. (MS-140) Cincinnati, Ohio 45268 fax number, (513) 569-7191 e-mail address: miller.carrie@epa.gov
- Step 2. Application The EPA may evaluate State and EPA Regional laboratory applications, to the extent resources permit, for sufficient equipment, experience, and demonstration of capability. Any deficiencies should be corrected before proceeding to the next step in the evaluation process.
    - Application Cover Letter (found at http://www.epa.gov/safewater/disinfection/lt2/pdfs/lab_qa_application_letter.pdf)
    - Application Package (found at http://www.epa.gov/safewater/disinfection/lt2/pdfs/lab_qa_application.pdf)
- Step 3. Initial proficiency test. After an application has been accepted, the laboratory will be sent a set of eight initial proficiency test (IPT) samples consisting of a suspension of oocysts in a concentrated matrix. Laboratories will resuspend these spikes in reagent water to produce simulated source water samples and analyze the samples using the version of Method 1622/1623 that the laboratory plans to use for routine *Cryptosporidium* analyses. If a laboratory wishes to be evaluated for more than one version of the method, the laboratory will receive a set of eight IPT samples for each version. Laboratory IPT data will be evaluated against mean recovery and precision (as relative standard deviation) criteria for the IPT samples.
- Step 4. On-site evaluation. After a laboratory passes the IPT and has documented the required capability to participate in the Lab QA Program through the completed application, an on-site evaluation of the laboratory will be scheduled. The on-site evaluation will include two separate, but concurrent, assessments: (1) assessment of the laboratory's sample processing and analysis procedures, including microscopic examination; and (2) evaluation of the laboratory's personnel qualifications, quality assurance and quality control, equipment, and record keeping procedures. After a

laboratory has corrected any deficiencies noted in the audit, they will be granted approval, and then will be listed with other laboratories that have passed the on-site evaluation on this site.

  - List of approved laboratories (found at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html#listapprovedlabs)

- Step 5. On-going Proficiency Test. EPA will evaluate on-going precision and recovery data to determine if the laboratory continues to meet the performance criteria of the Lab QA Program. Laboratories in the program will receive a set of three ongoing proficiency test samples approximately every four months that must be analyzed in the same manner as the IPT samples.

APPENDIX G

Application for Verification of *Cryptosporidium* Laboratory QA

OMB Control Number: 2040-0246
Expiration Date: 05/31/09

Application Package for Verification of *Cryptosporidium* Laboratory Quality Assurance

Submit electronic package to: miller.carrie@epa.gov

Please Note: A decision by the Agency to review this application, conduct an on-site evaluation, data evaluation or other activities, does not ensure that the review process will be completed. Decisions regarding the impact on a laboratory's approval status will be made based on the facts associated with a particular case, and actions will be taken as Agency resources permit.

Step 1: Submit all parts of this Application including Standard Operating Procedures and Record requests

In order to save the laboratory and EPA's time, please submit this all requested information in one package. Your application will be evaluated for completeness. Electronic versions are preferred whenever possible. Submit any necessary hard copies to: EPA Contractor for *Cryptosporidium* Laboratory QA Program**,** c/o Allie Bridges**,** 6101 Stevenson Avenue, Suite 500**,** Alexandria, VA 22304

Step 2: Schedule date to submit positive control and OPR slides

Submit an ongoing precision and recovery (OPR) microscope slide and the associated positive control slide for each method version used by your laboratory. Bench sheets and examination forms should be included with the slides. Submission will need to be coordinated with EPA contractor, CSC, pklonicki@csc.com or 513-563-6331 x2222.

Step 3: Order blind count slides for all analysts

Order a blind slide spiked with *Cryptosporidium* and *Giardia* for each analyst (Wisconsin State Laboratory of Hygiene 608-224-6260, or equivalent vendor.) Each analyst must perform an independent count of one slide. Submit slides and results using the Slide Count Report form (located at the Lab QA Web site http://www.epa.gov/safewater/disinfection/lt2/lab_home.html) to EPA contractor, CSC, 4701 Creek Road Suite 250, Cincinnati, OH 45242. Each laboratory should order at least one extra slide to cover any quality issues resulting from shipping.

Step 4: Internet Analyst Verification and Characterizations

A telephone/internet conference will be scheduled for the laboratory to demonstrate verification of performance for all microscopists. Please contact EPA contractor, CSC, pklonicki@csc.com or 513-563-6331 x2222 to schedule.

Step 5: On-site Visit and Order Blind Spikes

A day long on-site evaluation will focus on data recording and performance of Method 1623 with samples spiked with blind oocyst suspensions in the presence of an auditor. Bench sheets and examination forms for the blind samples, and associated method blank, and OPR samples should be submitted to EPA Contractor for *Cryptosporidium* Laboratory QA Program, c/o Allie Bridges, 6101 Stevenson Avenue, Suite 500, Alexandria, VA 22304. Please contact EPA contractor, CSC, abridges3@csc.com or 703-461-2411 to schedule. Order the blind samples (Wisconsin State Laboratory of Hygiene 608-224-6260, or equivalent vendor) after the on-site date has been scheduled.

Step 6: Evaluation

The laboratory will receive a report detailing all audit findings. The laboratory should provide complete written responses to any deficiencies or recommendations identified in the report within 60 days. Laboratory status for continued approval will be based on submission of acceptable responses, proficiency test results, quality of the positive control and OPR slide, slide counts, internet analyst verification, on-site evaluation, and recovery values for blind samples initiated during audit.

Laboratory Audit Score (Official Use Only)

Task	EPA Score			Date Completed
Application Package				
Positive Control and OPR Slides	**HV**	C -	G -	
	FM	C -	G -	
Blind Slide Count	Pass		Fail	
Internet Microscopy Verification				
On-Site Audit				
Method Holding Times Met	Pass		Fail	
Recovery of Blind IMS control Sample	C -		G -	
Recovery of On-site Blind Spike	**HV**	C -	G -	
	FM	C -	G -	
GLP – Personnel				
GLP – Equipment/Reagents				
GLP – SOPs				

Part 1. Laboratory Information

Laboratory Name:		
Address:		
City:	**State:**	**Zip:**
Contact Person:		
Title:		
Telephone:	**Fax:**	
Email address:		
Type of laboratory (check one): ☐ **Commercial** ☐ **Utility** ☐ **State** ☐ **Academic** ☐ **Other**		
Number of field samples your laboratory is analyzing per month using Method 1622/1623:		
Date of Previous Audit:	**Date of Initial Approval:**	

Part 2. Personnel List

Name of Current Analyst and Technicians	Current position (Principal Analyst, Analyst, or Technician)	Evaluated during Initial Audit or Documentation Submitted to EPA (Yes/No)

Part 3. Method and Equipment Information: Versions of Method 1623 for which the lab is seeking evaluation

Method 1623 Procedure		Key Equipment	Manufacturer/Model
Check all that apply		**Provide manufacturer and model for relevant pieces of equipment**	
Filtration			
Indicate the volume filtered for each		Cubitainer	
Pall Envirochek® HV		Pump	
IDEXX Filta-Max®		Flow control valve	
IDEXX Filta-Max xpress®		Flow meter or graduated container	
CFC		Filta-Max® housing	
Other (describe)		CFC bowl	
Elution			
Wrist action shaker		Laboratory shaker and side arms	
Stomaching of Filta-Max® filter		Stomacher	
Filta-Max® wash station		Filta-Max® Manual station	
Filta-Max xpress®		Filta-Max® Automatic station	
Other (describe)		IDEXX xpress® software version	
Concentration			
Centrifugation		Centrifuge - 1500 X G, swinging-bucket centrifuge for 15 mL - 250-mL tubes	
Filtration through membrane			
Other (describe)		Concentrator apparatus	
Purification			
Dynabeads® Crypto		Flat-sided sample tubes	
Dynabeads® CG-combo		Sample mixer/rotator for 10-mL tubes	
Other (describe)		Magnetic particle concentrator for 10-mL tubes	
		Magnetic particle concentrator for 1.5-mL tubes	
Staining and Examination			
Waterborne AquaGlo™		Microscope - Epifluorescence/ differential interference contrast (HMO or DIC) microscope with stage and ocular micrometers	
Waterborne Crypt-a-Glo™			
Waterborne Giardi-a-Glo™		20X to 100X objectives	
Meridian Merifluor®		Excitation/band pass microscope filters for fluorescein isothiocyanate (FITC) assay	
BTF EasyStain™			
Other (describe)		Excitation/band-pass filters for 4',6-diamidino-2-phenylindole (DAPI) assay	
Other			
Descriptions of “other” method steps and other comments:		Refrigerator for sample storage	
		Refrigerator for reagent storage	

Part 4. Standard Operating Procedures and Records

Electronic versions are preferred when possible, but photocopies are acceptable when electronic versions are not

possible. Well organized submissions, in order as listed below, will expedite processing.

A. Up-to-date standard operating procedures:

1. For each method step and version including: spiking, filtration, elution, concentration, purification, slide preparation, staining and examination
2. Dividing pellets greater than 0.5 mL
3. Preparation of reagents
4. Glassware washing
5. Staff training
6. Corrective action procedures for failing to meet OPR, method blank, staining controls, sample acceptance, and analyst verification criteria
7. Sampling procedures to be followed by field or utility personnel
8. Procedures for data recording, checking manual calculations, and checking accuracy of all data transcriptions
9. Procedures for electronic storage of data, including checking accuracy of data entry and backup of stored data

B. Ongoing Record Keeping

1. Training records for all analysts/technicians past and present
2. OPR control chart including at a minimum the last 20 OPR samples processed (See example template located at the Lab QA Web site http://www.epa.gov/safewater/disinfection/lt2/lab_home.html)
3. MS control charts including at a minimum the last 20 MS samples processed (See example template located at the Lab QA Web site http://www.epa.gov/safewater/disinfection/lt2/lab_home.html)

C. NELAC certificate (as applicable) as documentation of equipment maintenance

The above information is complete and accurate to the best of my knowledge.

_____

Name and Signature of Laboratory Director or Designee **Date**

Verification of *Cryptosporidium* Laboratory Quality Assurance
Checklist A – Audit Package and Data Review

Laboratory Name	Name and Affiliation of Evaluator	Date of Evaluation

Good Laboratory Practice (GLP) is generally defined as a system of management controls for the laboratories to ensure the consistency and reliability of results. Adapted from other federal programs for the purposes of the *Cryptosporidium* Laboratory QA Evaluation Program, GLP includes personnel, equipment, and standard operating procedures appropriate for the program.

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
1 Quality Assurance				
1.1 Has all documentation (e.g., resume, sample list) been submitted for all Method 1623 staff that have been added since the previous audit? [Section 9.1]	Requirement GLP			
1.1.1 Have technicians/analysts analyzed the required number of samples using Method1622/1623? [Section 22.2]	Requirement GLP			
1.2 Are employee training records available and up to date? [Section 9.1]	Critical GLP			
1.3 Is the laboratory performing analyst verification of examination monthly and does the lab have corrective action procedures in place if criteria are not met? [Section 10.6]	Requirement			
1.3.1 If the laboratory has only one analyst, is the analyst demonstrating analyst verification through comparison with photolibraries or repetitive counts?	Recommendation			
1.4 Does the quality assurance plan specifically address requirements for protozoa analysis under the LT2 program?	Critical			
1.5 Have acceptable initial precision and recovery analyses been performed for each version of the method the laboratory is using? [Section 9.1.2.1.1]	Requirement			
1.6 Of the 10 field samples reviewed and their associated method blanks, is each field sample associated with an acceptable method blank? [Section 9.6.1]	Requirement			
1.6.1 Were all method blanks evaluated without contamination?	Requirement			

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
1.6.2 Were the same lots of reagents (elution, IMS, and staining) used for the method blank and the associated field samples?	Critical			
1.6.3 Is method blank analyzed prior to the analysis of field samples? [Section 9.6]	Requirement			
1.6.4 How many method blanks were evaluated?				
1.7 Is each field sample associated with an acceptable ongoing precision and recovery (OPR) sample? [Section 9.7]	Requirement			
1.7.1 What percentage of OPR samples evaluated met the recovery criteria? [Table 3; Section 9.7.3]				
1.7.2 Were the same lots of reagents (elution, IMS, and staining) used for the OPR and the associated field samples?	Critical			
1.7.3 Is OPR analyzed prior to the analysis of field samples? [Section 9.7]	Requirement			
1.7.4 Does the laboratory maintain control charts of OPR results? [Section 9.7.6] If not, how do they measure method performance per Section 9.1?	Recommendation			
1.7.5 How many OPR samples were evaluated?				
1.7.6 What is the mean and relative standard deviation (RSD), or standard deviation, of the recoveries of the OPR samples included in the control chart?				
1.8 Were matrix spike (MS) samples analyzed at the method - specified frequency? [Section 9.1.8]	Requirement			
1.8.1 How many MS samples were evaluated?				
1.8.2 Were MS sample volumes within 10% of their associated field samples' volumes? [Section 9.5.1]	Requirement			
1.8.3 Were MS samples analyzed at the same time and using the same method variation as their associated field samples?	Requirement			
1.8.4 What is the mean and relative standard deviation of the MS samples reviewed?				
1.8.5 Does the laboratory maintain control charts of MS results? If not, how do they measure method performance per Section 9.1?	Recommendation			
1.9 Were OPR and MS samples spiked with 100 - 500 organisms? [Section 9.7]	Requirement			

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
1.9.1 If the answer to 1.9 is no, then at what level were samples spiked?				
1.10 Does the laboratory perform IMS controls and maintain IMS control charts? If not, how do they troubleshoot low recoveries?	Recommendation			
1.11 Are the laboratory personnel performing the QC analyses representative of the personnel seeking approval under this program?	Critical			
1.12 Does the laboratory have an adequate record system for tracking samples from collection through log-in, analysis, and data reporting?	Critical GLP			
1.13 Is the laboratory using the December 2005 version of Method 1622/1623 for LT2 samples? [CFR 40 Part 141.704]	Requirement			
2 Data Recording Procedures				
2.1 Is shipping information complete, including the time and date of sample collection, sampler's name, time and date of sample receipt, sample condition, and noting any discrepancies between samples on the traffic report and samples received? [Section 8.1.3]	Requirement			
2.1.1 Were all samples evaluated received at ≤20°C and not frozen? [Section 8.1.3]	Requirement			
2.2 Do sample numbers on the chain of custody match the sample numbers on the report forms?	Requirement			
2.3 Are current Method 1622/1623 bench sheets used to record sample processing data?	Recommendation			
2.4 Are all primary measurements during each step recorded, including all raw data used in calculations? [Section 11.0, 12.0, 13.0]	Requirement			
2.5 Name of analyst or technician performing the elution is recorded?	Critical			
2.6 Date and time of elution is recorded? [Section 12.2.6.2.1]	Requirement			
2.7 Name of analyst or technician performing the slide preparation is recorded?	Critical			
2.8 Date and time of slide preparation is recorded? [Section 13.3.3.11]	Requirement			
2.9 Name of analyst or technician performing the staining is recorded?	Critical			
2.10 Date and time of staining is recorded? [Section 14.10]	Requirement			

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
2.11 Are batch and lot numbers of reagents used in the analysis of the sample recorded?	Critical			
2.12 Lot number for the IMS kit is recorded?	Critical			
2.13 Lot number of the staining kit is recorded?	Critical			
2.14 Lot number of the spiking suspensions is recorded?	Critical			
2.15 Spike value recorded for all spiked samples?	Requirement			
2.16 Are Method 1622/1623 *Cryptosporidium* Slide Examination forms used to record sample examination results? [Section 15.2]	Requirement			
2.17 Name of examining analyst is recorded? [Section 15.2.6]	Requirement			
2.18 Date and time of sample examination is recorded? [Section 15.2.4]	Requirement			
2.19 Are calculations of final concentrations and recoveries complete and correct?	Requirement			
2.20 Is the size of the cysts and oocysts reported to the nearest 0.5 µm? [Section 15.2.2.3]	Requirement			
2.21 Do values recorded on the data sheets match the values reported to the LT2 DCTS?	Requirement			
2.22 Do values recorded on the data sheets match the values reported to the client?	Requirement			
2.23 Are mistakes on all forms crossed out with a single line, initialed, and dated?	Critical			
2.24 Are data always legible and recorded in pen?	Critical			
2.25 Was the final report reviewed by QA manager, lab director or an individual other than the analyst?	Critical			
2.26 Do records demonstrate each analyst's characterization of 3 oocysts and 3 cysts from positive control for each microscopy session? [Section 15.2.1.1]	Requirement			
2.27 Data shows that no more than 0.5 mL of pellet was used per IMS? [Section 13.2.4]	Requirement			
3 Holding Times – December 2005 version of Method 1622/1623				
3.1 Is sample elution initiated within 96 hours of sample collection or field filtration? [Section 8.2.1]	Requirement			
3.2 Are sample elution, concentration, and purification steps completed in one work day? [Section 8.2.2]	Requirement			

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
3.3 Are slides stained within 72 hours of application of the purified sample to the slide? [Section 8.2.3]	Requirement			
3.4 Are stained slides read and confirmed within 7 days of staining? [Section 8.2.4]	Requirement			
4 Spike enumeration procedures				
4.1 Source of oocysts for spikes				
4.2 If 50-L samples are analyzed, what positive control procedure does the laboratory follow for OPR and MS samples: (A) spike entire 50 L, (B) spike and filter 10 L before filtering 40 L, or (C) filter 40 L before spiking and filtering 10 L.				
The following items below are optional if the laboratory is NELAC certified. If the laboratory opts to provide NELAC certification, complete the box below by entering the NELAC certification number and date. Provide copy of certification.				
NELAC Certification Number:		**Certification Date:**		
5 Laboratory Equipment and Supplies				
5.1 Reagent-grade water testing				
5.1.1 Is reagent water tested monthly for conductivity and total chlorine residual?	Critical GLP			
5.1.1.1 Were the results for the above parameters acceptable? Total chlorine residual not greater than 0.1 mg/L, conductivity not greater than 2 µmhos/cm?	Critical GLP			
5.1.2 Has the reagent water been tested annually for metals – Pb, Cd, Cr, Cu, Ni, Zn?	Critical GLP			
5.1.2.1 Were the results for the metals testing acceptable; each metal not greater than 0.05 mg/L and collectively not greater than 0.1 mg/L?	Critical GLP			
5.1.3 Is reagent water tested monthly for heterotrophic plate count?	Critical GLP			
5.1.3.1 Are the results for the heterotrophic plate count acceptable, < 500 CFU/mL?	Critical GLP			
5.1.4 Is still or DI unit maintained according to manufacturer's instructions?	Critical GLP			
5.2 pH meter				
5.2.1 Accuracy ± 0.1 units, scale graduations, 0.1 units?	Critical GLP			

Item to be Evaluated		Classification	Yes, No, NA, or Unknown	Comments	Response Requested
5.2.2	Is a record maintained for pH measurements and calibrations?	Critical GLP			
5.2.3	Is pH meter standardized each use period with pH 7, 4 or 10 standard buffers (selection dependent upon desired pH)?	Critical GLP			
5.2.4	Are all pH buffers dated when received and opened, and discarded before expiration date?	Critical GLP			
5.3	**Balances (top loader or pan balance)**				
5.3.1	Are balances calibrated monthly using ANSI/ASTM Class 1, Class 2 or Class 3 weights or weights traceable to Class 1, Class 2, or Class 3 weights, or equivalent? Non reference weights should be calibrated every six months with reference weights.	Critical GLP			
5.3.2	Is correction data and Certificate of Traceability available for weights?	Critical GLP			
5.3.3	Is preventative maintenance conducted yearly at a minimum?	Recommendation GLP			
5.4	**Temperature recording device**				
5.4.1	Are calibration of thermometers checked annually (dial thermometers quarterly) at the temperature used against a reference NIST thermometer or equivalent? [Section 8.1.4]	Requirement GLP			
5.4.2	Is the sample storage refrigerator able to maintain temperature of 1 to 10°C?	Critical GLP			
5.5	**Micropipetters**				
5.5.1	Have micropipetters been calibrated within the past year? [Section 9.2.1]	Requirement GLP			
5.6	**Centrifuge**				
5.6.1	Is a maintenance contract in place or internal maintenance protocol available? [Section 9.1]	Critical GLP			
5.6.2	Is the centrifuge calibrated yearly?	Critical GLP			
5.7	**Autoclave**				
5.7.1	Are date, contents, sterilization time and temperature, and technician initials recorded for each cycle?	Critical GLP			
5.7.2	Is a maximum registering thermometer or continuous monitoring device used during each autoclave cycle?	Critical GLP			

Item to be Evaluated	Classification	Yes, No, NA, or Unknown	Comments	Response Requested
5.7.3 Is automatic timing mechanism checked with stopwatch quarterly?	Critical GLP			
5.7.4 Are spore strips or ampules used monthly to confirm sterilization?	Critical GLP			
6 Quality Assurance Manual				
6.1 Does the laboratory have a formal QA laboratory plan prepared and ready for examination? [Section 9.1]	Requirement			
6.2 Is a laboratory organization chart or other information available listing staff organization and responsibilities? Does it identify the QA manager and lab director?	Recommendation			
6.2.1 Is the QA manager separate from the lab director?	Recommendation GLP			
6.3 Does the laboratory have a checklist or calendar of preventative maintenance procedures and schedules? [Section 9.1]	Requirement GLP			

Laboratory Name	Name and Affiliation of Evaluator	Date of Evaluation

Good Laboratory Practice (GLP) is generally defined as a system of management controls for the laboratories to ensure the consistency and reliability of results. Adapted from other federal programs for the purposes of the *Cryptosporidium* Laboratory QA Evaluation Program, GLP includes personnel, equipment, and standard operating procedures appropriate for the program.

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
1 Sample Spiking				
1.1 The suspension vial is vortexed for 30 seconds or per manufacturer's instructions? [Section 11.4.3]	Method Procedure			
1.2 The carboy used for the method blank is randomly selected from carboy stock to check efficacy of cleaning system or disposable carboys are used for all samples?	Critical			
1.3 The details of the suspension vial rinse, including volumes? [Section 11.4.3.1]	Method Procedure			
1.4 Acceptable sample spiking procedures, including issues not noted in items 1.1 through 1.3?	Critical GLP			
2 Filtration/Elution				
2.1 Envirochek® filtration				
2.1.1 The flow rate is maintained at approximately 2 L/min? [Section 12.2.1.2]	Method Procedure			
2.1.2 The volume filtered is measured using a flow totalizer or calibrated carboy? [Section 12.2.4.2]	Requirement			
2.1.3 The sample is stirred during filtration? [Section 12.2.4.1]	Method Procedure			
2.1.4 The details of the carboy rinse after filtration including volume? [Section 12.2.4.5]	Method Procedure			
2.1.5 Appropriate maintenance and cleaning procedures?	Critical			
2.1.6 Acceptable Envirochek® filtration procedures, including issues not noted in items 2.1.1 through 2.1.5?	Critical GLP			
2.2 Envirochek® capsule filter elution				

Item to be Evaluated For each item, does the SOP specify:		Classification	Yes, No, NA or Unknown	Comments	Response Requested
2.2.1	Measurement of the volume of the elution buffer used or that the volume covers the membrane? [Section 12.2.6.2.2]	Method Procedure			
2.2.2	The speed that samples are shaken? [Section 12.2.6.2.3]	Method Procedure			
2.2.3	The samples are shaken three times for 5 minutes each time, and each in a different orientation? [Section 12.2.6.2]	Method Procedure			
2.2.4	Procedures for filter capsule rinse and addition of rinsate to the centrifuge bottle? [Section 12.2.6.2.8]	Method Procedure			
2.2.5	Acceptable Envirochek® capsule filter elution procedures, including issues not noted in items 2.2.1 through 2.2.4?	Critical GLP			
2.3 Filta-Max® filtration					
2.3.1	The flow rate is maintained at <4 L per minute for Filta-Max® or <2 L per minute for Filta-Max xpress®? [Section 12.3.1.1.3 or manufacturer's instructions]	Method Procedure			
2.3.2	The volume filtered is measured using a flow totalizer or calibrated carboy? [Section 12.3.1.5.2]	Requirement			
2.3.3	Appropriate maintenance and cleaning procedures? [Section 12.3.4]	Requirement			
2.3.4	Acceptable Filta-Max® filtration procedures, including issues not noted in items 2.3.1 through 2.3.3?	Critical GLP			
2.4 Filta-Max® filter wash station elution					
2.4.1	The use of PBST to elute the filter? [Section 7.4.2.4]	Method Procedure			
2.4.2	The amount of PBST used for each wash? (approx. 600 mL) [Section 12.3.2.2.1]	Method Procedure			
2.4.3	The plunger is moved up and down 20 times during the first wash? [Section 12.3.2.2.1]	Method Procedure			
2.4.4	The plunger is moved up and down gently to avoid generating excess foam?	Method Procedure			

Item to be Evaluated For each item, does the SOP specify:		Classification	Yes, No, NA or Unknown	Comments	Response Requested
2.4.5	That during the second wash the plunger is moved up and down 10 times? [Section 12.3.2.2.2]	Method Procedure			
2.4.6	The instructions for cleaning the wash station between samples? [Section 12.3.4.2]	Requirement			
2.4.7	The housing is rinsed after filter is removed and the rinse is included in the sample volume? [Section 12.3.2.2]	Method Procedure			
2.4.8	Acceptable Filta-Max® filter wash station elution procedures, including issues not noted in items 2.4.1 through 2.4.7?	Critical GLP			
2.5 Filta-Max® filter stomacher elution					
2.5.1	The use of PBST to elute the filter? [Section 7.4.2.4]	Method Procedure			
2.5.2	The amount of PBST used for each wash? (approx. 600 mL) [Section 12.3.2.3]	Method Procedure			
2.5.3	Two washes are performed for 5 minutes each? [Section 12.3.2.3]	Method Procedure			
2.5.4	The housing is rinsed after filter is removed and included in the sample volume?	Critical			
2.5.5	Acceptable Filta-Max® filter stomacher elution procedures, including issues not noted in items 2.5.1 through 2.5.4?	Critical GLP			
2.6 Filta-Max xpress® elution system					
2.6.1	The correct set up of the pressure elution station?	Method Procedure			
2.6.2	A system check is performed prior to sample elution?	Method Procedure			
2.6.3	PBST elution buffer is used to elute the filter?	Method Procedure			
2.6.4	The buffer reservoir contains the appropriate amount of buffer? (400 mL for initial system check and 400 mL for each subsequent elution)	Method Procedure			
2.6.5	The appropriate pressure regulator setting [high pressure gauge should read ~72.5 psig, while low pressure gauge should read ~5.5 psig]?	Method Procedure			
2.6.6	The QC stem wash station is cleaned adequately between samples?	Requirement			

Item to be Evaluated For each item, does the SOP specify:		Classification	Yes, No, NA or Unknown	Comments	Response Requested
2.6.7	The buffer solution is purged with reagent water after the last sample was processed?	Method Procedure			
2.6.8	The system is maintained following manufacturer's instructions (i.e., daily, weekly, and monthly routine maintenance – air compressor drained, pressure chamber cleaned, etc.).	Method Procedure			
2.6.9	The system is serviced by a qualified IDEXX service technician every 10,000 cycles or when system alerts user?	Method Procedure			
2.6.10	Acceptable Filta-Max xpress® elution system procedures, including issues not noted in items 2.6.1 through 2.6.9?	Critical GLP			
3 Concentration					
3.1 Filta-Max® filter sample concentration (as an alternative or in addition to Section 3.2)					
3.1.1	The force of the vacuum is maintained below 30 cm Hg? [NOTE, pg 43]	Method Procedure			
3.1.2	That concentration is performed after each of the washes?	Method Procedure			
3.1.3	The sample is concentrated so that some liquid remains above the filter (enough to cover the stir bar about half-way)? [Section 12.3.3.2.1]	Method Procedure			
3.1.4	The stir bar and concentration tube are rinsed after each concentration and the liquid added to the concentrate? [Section 12.3.3.2.1]	Requirement			
3.1.5	The filter membrane is washed twice? [Section 12.3.3.2.3]	Method Procedure			
3.1.6	That 5 mL of PBST is used each time? [Section 12.3.3.2.3]	Method Procedure			
3.1.7	Acceptable Filta-Max® filter sample concentration procedures, including issues not noted in items 3.1.1 through 3.1.6?	Critical GLP			
3.2 Envirochek® and Filta-Max® filter sample centrifugation					
3.2.1	The sample is centrifuged at 1500 x G (maximum 2000 x G) using a swinging bucket rotor? [Section 13.2.1, NOTE, pg 46]	Method Procedure			

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
3.2.2 Instructions to ensure the centrifuge tubes are properly balanced prior to centrifugation?	Critical			
3.2.3 The sample is centrifuged for 15 minutes? [Section 13.2.1]	Method Procedure			
3.2.4 The centrifugation start time begins when centrifuge reaches the required speed?	Method Procedure			
3.2.5 The centrifuge is slowly decelerated at the end without using the brake? [Section 13.2.1]	Method Procedure			
3.2.6 Acceptable Envirochek® and Filta-Max® filter sample centrifugation procedures, including issues not noted in items 3.2.1 through 3.2.5?	Critical GLP			
4 Purification and Slide Preparation				
4.1 The centrifuged sample supernatant is aspirated no lower than 5 mL above the pellet or no lower than 8 mL for Filta-Max xpress®? [Section 13.2.2 or manufacturer's instructions]	Requirement			
4.1.1 The type and internal diameter of pipette used for aspiration of supernatant?	Recommendation			
4.1.2 The rate of aspiration (i.e., mL/ min or pressure of the vacuum)?	Recommendation			
4.2 The tube is vortexed vigorously until pellet is completely resuspended? [Section 13.2.3]	Method Procedure			
4.3 Appropriate procedures for dividing pellets greater than 0.5 mL into subsamples and the analysis of the subsamples?	Critical			
4.4 That no more than 0.5 mL of pellet is used per IMS? [Section 13.2.4]	Method Procedure			
4.5 The resuspended pellet volume is quantitatively transferred to the flat-sided tube (2 rinses) including the determination of the rinse volumes? [Section 13.3.2.1]	Method Procedure			
4.6 That SL-Buffer A is used at room temperature or that it is checked for precipitate before use? [NOTE, pg 47]	Method Procedure			
4.7 That the volume of 10x SL-Buffer A is 1 mL? [Section 13.3.1.2]	Method Procedure			
4.8 That the volume of 10x SL-Buffer B is 1 mL? [Section 13.3.1.3]	Method Procedure			

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.9 Instructions for thorough resuspension of IMS beads prior to addition to the flat-sided tube? [Section 13.3.2.2]	Method Procedure			
4.10 100 µL of *Cryptosporidium* and *Giardia* beads are used? [Section 13.3.2.3 and 13.3.2.5]	Method Procedure			
4.11 The flat-sided tube is rotated at 18 rpm for 1 hour at room temperature? [Section 13.3.2.6]	Method Procedure			
4.12 Which magnetic concentrators, MPC®-1 or MPC®-6, are used?	Method Procedure			
4.13 The placement of the flat-sided tube in the magnet and the rock technique and time? [Section 13.3.2.9]	Method Procedure			
4.14 The sample is quantitatively transferred from the flat-sided tube to the microcentrifuge tube (2 rinses) including rinse volumes? [Section 13.3.2.13]	Method Procedure			
4.15 The flat-sided tube is allowed to sit one minute after each transfer to accumulate residual sample, then the residual is transferred to microcentrifuge tube?	Method Procedure			
4.16 The position of the magnet in the MPC®-S?	Method Procedure			
4.17 The types of extra rinses performed to minimize debris; and how laboratory performs the extra rinse? A) IMS beads in the flat-sided tube prior to transfer B) Flat-sided tube, not IMS beads, prior to transfer C) IMS beads in microcentrifuge tube prior to dissociation?	Method Procedure	Circle one: A B C		
4.18 Criteria for determining when extra rinses should be performed, if extra rinses are not performed for all samples?	Recommendation			
4.19 The technique for the extra rinse including volumes and reagents used?	Method Procedure			
4.20 That standard NaOH (5 µL, 1N) and standard HCl (50 µL, 0.1N) are used? [NOTE, pg 49]	Requirement			
4.21 The sample is vortexed vigorously for 50 seconds immediately after the addition of acid and 30 seconds after the sample has set for 10 minutes at room temperature? [Section 13.3.3]	Method Procedure			

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.22 A second dissociation is performed? [Section 13.3.3.10]	Requirement			
4.23 When the second dissociation is performed, the laboratory: A) uses a second slide, or B) adds the additional volume to the original slide? [Section 13.3.3.10]		Circle one: A B		
4.24 The volume and the timing of the NaOH addition to the wells? [Section 13.3.3.8]	Method Procedure			
4.25 When the slides are dried (e.g., room temperature or slide warmer), the laboratory: A) uses room temperature [Section 13.3.3.12], or B) uses 35 to 42 C [Section 13.3.3.12], or C) follows manufacturer's instructions?		Circle one: A B C		
4.26 If the laboratory has more than one option specified for slide drying, are criteria included for when each option will be used?	Recommendation			
4.27 That positive and negative staining controls are prepared at the same time the slides are prepared? [Section 14.1]	Requirement			
4.28 Acceptable sample purification and slide preparation procedures, including issues not noted in items 4.1 through 4.27?	Critical GLP			
5 Sample Staining				
5.1 Which stain to use and to follow manufacturer's instructions for FITC stain application? [Section 14.2]	Method Procedure			
5.2 The slides are incubated in a humid chamber in the dark at room temperature for approximately 30 minutes or per manufacturer's directions? [Section 14.3]	Method Procedure			
5.3 The working DAPI stain is prepared the day it is used? [Section 7.7.2]	Method Procedure			
5.4 The stock DAPI is stored at 1 to 10°C in the dark? [Section 7.7.1]	Method Procedure			
5.5 The volume of working DAPI applied and the incubation time? [Section 14.6]	Method Procedure			
5.6 The technique used to drain the excess stain from the well and to rinse the well?	Method Procedure			

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
5.7 What type and amount of mounting media used?	Method Procedure			
5.8 That all the edges of the cover slip are sealed well with clear fingernail polish, unless Elvenol is used? [Section 14.9]	Method Procedure			
5.9 The finished slides or slides not read immediately are stored in a humid chamber in the dark at 1° to 10°C (humid chamber not required for Evenol)? [Section 14.10]	Method Procedure			
5.10 Acceptable sample staining procedures, including issues not noted in items 5.1 through 5.9?	Critical GLP			
6 Microscope and Examination				
6.1 Instructions for Kohler and ocular adjustments? [Sections 10.3.4 and 10.3.6]	Requirement			
6.2 That all measurements must be recorded to the nearest 0.5 micron? [Section 15.2.2.3]	Requirement			
6.3 Microscope cleaning procedures? [Section 10.4]	Requirement			
6.4 The recording of coordinates of all cysts and oocysts on the worksheet for future reference; and slide orientation on the microscope stage to standardize coordinate recording?	Recommendation			
6.5 The examination and acceptance of positive and negative staining controls before proceeding with examination of field samples? [Section 15.2.1]	Requirement			
6.6 That each analyst characterizes 3 oocysts and 3 cysts on the positive staining control at each examination session? [Section 15.2.1.1]	Requirement			
6.7 Corrective actions if positive and/or negative staining controls are not acceptable?	Recommendation			
6.8 The criteria for organism identification? [Section 15.2.2]	Requirement			
6.9 Acceptable microscope and examination procedures, including issues not noted in items 6.1 through 6.8?	Requirement GLP			
7 Reagents				
7.1 Procedures for the preparation of all essential chemicals and reagents?	Critical			
7.2 That expiration dates are specified for all reagents prepared by the laboratory?	Critical			
8 Quality Assurance				

Item to be Evaluated For each item, does the SOP specify:	Classification	Yes, No, NA or Unknown	Comments	Response Requested
8.1 Training protocol for new employees? [Section 9.1]	Requirement GLP			
8.2 Procedures for performing analyst verification? [Section 10.6]	Requirement GLP			
8.3 Acceptable procedures for sample collection for field or utility personnel?	Critical GLP			
8.4 Criteria for sample acceptance and corrective action procedures? [Section 8.1.3]	Requirement GLP			
8.5 Manual data recording procedures?	Critical GLP			
8.6 Procedures for checking the accuracy of data transcriptions, including electronic data entry?	Critical GLP			
8.7 Procedures for checking the accuracy of manual calculations?	Critical GLP			
8.8 Procedures for electronic data entry and storage?	Critical GLP			
8.9 How backup of stored data is performed?	Critical GLP			
8.10 Corrective action procedures for OPR failures? [Section 9.7.4]	Requirement GLP			
8.11 Corrective action procedures for method blank contamination? [Section 9.6.2]	Requirement GLP			
8.12 Procedures for identifying and assessing declining trends in recovery through review of control charts and/or other recovery data?	Recommendation GLP			
8.13 Corrective action procedures for investigating QC failures or declining trends in recovery?	Recommendation GLP			
8.14 Acceptable glassware washing procedures?	Critical GLP			

Verification of *Cryptosporidium* Laboratory Quality Assurance
Checklist C – Technical Review – Sample Processing and Microscopy

Laboratory Name	Name and Affiliation of Evaluator	Date of Evaluation

Good Laboratory Practice (GLP) is generally defined as a system of management controls for the laboratories to ensure the consistency and reliability of results. Adapted from other federal programs for the purposes of the *Cryptosporidium* Laboratory QA Evaluation Program, GLP includes personnel, equipment, and standard operating procedures appropriate for the program.

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
1 Laboratory Facilities				
1.1 Does laboratory appear to have established appropriate safety and health practices prior to use of this method?	Critical			
1.1.1 Are references for safety and health practices, GLP and QA/QC readily available to analysts? (At a minimum, references could include the documents in Method 1623 Section 20.)	Recommendation			
1.2 Do all laboratory personnel wear gloves when handling biohazard and toxic compounds, and change gloves before touching other surfaces and equipment?	Critical GLP			
1.3 Does the laboratory disinfect bench surfaces before and after analyses?	Critical GLP			
1.4 Does the laboratory have adequate bench space to perform the method?	Critical GLP			
1.5 Other than the issues noted in items 1.1 through 1.4 (if any), no other facility issues were observed?				
2 Reagents				
2.1 Is reagent water used to prepare all reagents? [Section 7.3]	Requirement			
2.2 Are all reagents clearly labeled with identity of reagent, date of preparation, technician initials, and expiration date?	Critical GLP			
2.3 Are SOPs available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
3 Sample Spiking		**Technician:**		
3.1 If flow-sorted spikes are used, was suspension vial vortexed for 30 seconds or per manufacturer's instructions? [Section 11.4.3]	Method Procedure			
3.2 Is the carboy used for method blank randomly selected from carboy stock to check efficacy of cleaning system?	Critical GLP			
3.3 Was the suspension vial adequately rinsed? [Section 11.4.3.1]	Method Procedure			
3.4 Are SOPs for sample spiking available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
3.5 Other than issues noted for items 3.1 through 3.4 (if any) was sample spiking demonstrated successfully?				
4 Filtration/Elution				
4.1 Envirochek® filtration		**Technician:**		
4.1.1 Are all components required for sample filtration present and in good condition? [Section 6.2]	Requirement GLP			
4.1.2 Is the filter assembly set up correctly? [Figure 3a, pg 63]	Method Procedure GLP			
4.1.3 Is the pump adequate for needs? [Section 6.3.3]	Requirement GLP			
4.1.4 Is the appropriate flow rate maintained (approximately 2 L/min)? [Section 12.2.1.2]	Method Procedure			
4.1.5 Is the volume filtered measured using a flow totalizer or calibrated carboy? [Section 12.2.4.2]	Requirement			
4.1.6 Is the system well maintained and cleaned appropriately following use?	Critical GLP			
4.1.7 Is the system able to maintain seal during use with no leaks?	Requirement GLP			
4.1.8 Are SOPs for Envirochek® filtration available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.1.9 Other than issues noted for items 4.1.1 through 4.1.8 (if any) was Envirochek® filtration demonstrated successfully?				
4.2 Envirochek® capsule filter elution		**Technician:**		
4.2.1 Is the elution buffer prepared as per Method 1622/1623? [Section 7.4.1]	Method Procedure			
4.2.2 Is the wrist-shaker assembly set up correctly with arms fully extended? [Section 2.2.6.1.1]	Method Procedure GLP			
4.2.3 Does the eluting solution cover the membrane? [Section 12.2.6.2.2]	Method Procedure			
4.2.4 Is volume of elution buffer measured to ensure the use of one 250 mL centrifuge tube? [Section 12.2.6.2.2]	Method Procedure			
4.2.5 Are the samples shaken at an appropriate speed? [Section 12.2.6.2.3]	Method Procedure			
4.2.6 Are the samples shaken three times for 5 minutes each time, and each in a different orientation? [Section 12.2.6.2]	Method Procedure			
4.2.7 Are SOPs for Envirochek® capsule filter elution available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
4.2.8 Other than issues noted for items 4.2.1 through 4.2.7 (if any) was Envirochek® capsule filter elution demonstrated successfully?				
4.3 Filta-Max™ filtration		**Technician:**		
4.3.1 Which filter is used – Filta-Max® (black end caps) or Filta-Max xpress® (red end caps)?				
4.3.2 Are all components required for sample filtration present and in good condition? [Section 6.2.3]	Requirement GLP			
4.3.3 Is the filter assembly set up correctly? [Fig. 3b, pg. 64]	Method Procedure GLP			
4.3.3.1 Did the technician handle the filter without touching the foam?	Critical			

Item to be evaluated		Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.3.4	Is appropriate flow rate maintained of <4 L per minute for Filta-Max™ or <2 L per minute for Filta-Max xpress®? [Section 12.3.1.1.3 or manufacturer's instructions]	Method Procedure			
4.3.5	Is the volume filtered measured correctly using a flow meter or calibrated carboy? [Section 12.3.1.5.2]	Requirement GLP			
4.3.6	Is system well maintained and cleaned appropriately following use? [Section 12.3.4]	Requirement GLP			
4.3.7	Is system able to maintain seal during use with no leaks?	Requirement GLP			
4.3.8	Does the laboratory indicate on the filter housing the correct direction of flow? [Section12.3.1.3]	Critical			
4.3.9	Are SOPs for Filta-Max® filtration available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
4.3.10	Other than issues noted for items 4.3.1 through 4.3.9 (if any) was Filta-Max® filtration demonstrated successfully?				
4.4 Filta-Max® filter wash station elution			**Technician:**		
4.4.1	Is an automatic or manual wash station used?				
4.4.2	Is the filter wash station set up correctly? [Section 12.3.2.1]	Requirement GLP			
4.4.3	Is PBST used to elute the filter? [Section 7.4.2.4]	Method Procedure			
4.4.4	Is an appropriate amount of PBST used for each wash? (approx. 600 mL) [Section 12.3.2.2.1]	Method Procedure			
4.4.5	During the first wash, is the plunger moved up and down 20 times? [Section 12.3.2.2.1]	Method Procedure			
4.4.6	Is the plunger moved up and down gently to avoid generating excess foam?	Method Procedure			
4.4.7	During the second wash, is the plunger moved up and down 10 times? [Section 12.3.2.2.2]	Method Procedure			
4.4.8	Is residual suspension rinsed from all containers and gloves?	Critical			

Item to be evaluated		Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.4.9	If the automatic washer is used, is the machine operating properly? [Section 12.3.2.1]	Requirement			
4.4.10	Is the wash station cleaned adequately between samples? [Section 12.3.4.2]	Requirement GLP			
4.4.11	Are SOPs for Filta-Max® filter wash station elution available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
4.4.12	Other than issues noted for items 4.4.1 through 4.4.11 (if any) was Filta-Max® filter wash station elution demonstrated successfully?				
4.5 Filta-Max® filter stomacher elution			**Technician:**		
4.5.1	Is PBST used to elute the filter? [Section 7.4.2.4]	Method Procedure			
4.5.2	Is an appropriate amount of PBST used for each wash? (approx. 600 mL) [Section 12.3.2.3]	Method Procedure			
4.5.3	Are two washes performed for 5 minutes each? [Section 12.3.2.3]	Method Procedure			
4.5.4	Is the stomacher in good condition and operating properly?	Requirement GLP			
4.5.5	Is residual suspension rinsed from all containers and gloves?	Critical			
4.5.6	Are SOPs for Filta-Max® filter stomacher elution available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
4.5.7	Other than issues noted for items 4.5.1 through 4.5.6 (if any) was Filta-Max® filter stomacher elution demonstrated successfully?				
4.6 Filta-Max filter xpress® elution system			**Technician:**		
4.6.1	Is the pressure elution station set up correctly?	Requirement GLP			
4.6.2	Is a system check performed prior to sample elution?	Method Procedure			
4.6.3	Is PBST elution buffer used to elute the filter?	Method Procedure			

Item to be evaluated		Classification	Yes, No, NA or Unknown	Comments	Response Requested
4.6.4	Does the buffer reservoir contain the appropriate amount of buffer? (400 mL for initial system check and 400 mL for each subsequent elution)	Method Procedure			
4.6.5	Is the pressure regulator setting appropriate (high pressure gauge should read ~72.5 psig, while low pressure gauge should read ~5.5 psig)?	Method Procedure			
4.6.6	Is the QC stem wash station cleaned adequately between samples?	Requirement GLP			
4.6.7	Is the buffer solution purged with reagent water after the last sample was processed?	Method Procedure			
4.6.8	Is the system maintained following manufacturer's instructions (i.e., daily, weekly, and monthly routine maintenance – air compressor drained, pressure chamber cleaned, etc.)	Method Procedure GLP			
4.6.9	The system is serviced by a qualified IDEXX service technician every 10,000 cycles or when system alerts user?	Method Procedure GLP			
4.6.10	Are SOPs for Filta-Max filter xpress® elution system available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
4.6.11	Other than issues noted for items 4.6.1 through 4.6.10 (if any) was Filta-Max xpress® elution system demonstrated successfully?				
5	**Concentration**				
5.1	**Filta-Max® filter sample concentration (as an alternative to Section 5.2)**		**Technician:**		
5.1.1	Is concentrator set up correctly? [Section 12.3.3.2.1 b.]	Requirement GLP			
5.1.2	Is the force of the vacuum maintained below 30 cm Hg? [note, pg. 43]	Method Procedure			
5.1.3	Is concentration performed after each of the washes?	Method Procedure			
5.1.4	Is the sample concentrated so that some liquid remains above the filter (enough to cover the stir bar about half-way)? [Section 12.3.3.2.1 c.]	Method Procedure			

Item to be evaluated		Classification	Yes, No, NA or Unknown	Comments	Response Requested
5.1.5	Are the stir bar and concentration tube rinsed after each concentration and the liquid added to the concentrate? [Section 12.3.3.2.1 c.]	Requirement			
5.1.6	Was the filter membrane washed twice? [Section 12.3.3.2.3]	Method Procedure			
5.1.7	Was 5 mL of PBST used each time? [Section 12.3.3.2.3]	Method Procedure			
5.1.8	Is the membrane adequately washed to remove oocysts from filter?	Method Procedure			
5.1.9	Are SOPs for Filta-Max® filter sample concentration available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
5.1.10	Other than issues noted for items 5.1.1 through 5.1.9 (if any) was Filta-Max® filter sample concentration demonstrated successfully?				
5.2 Envirochek® and Filta-Max® filter sample centrifugation			**Technician:**		
5.2.1	Is the sample centrifuged at 1500 x G (maximum 2000 x G) using a swinging bucket rotor? [Section 13.2.1]	Method Procedure GLP			
5.2.2	Are the centrifuge tubes properly balanced prior to centrifugation?	Critical			
5.2.3	Does lab have easily accessible method for determining relative centrifugal force of centrifuges?	Critical GLP			
5.2.4	Is the sample centrifuged for 15 minutes? [Section 13.2.1]	Method Procedure			
5.2.5	Does the centrifugation time begin when the centrifuge reaches the desired speed? [Section 13.2.1]	Method Procedure			
5.2.6	Is the centrifuge slowly decelerated at the end without the brake? [Section 13.2.1]	Method Procedure			
5.2.7	Is the pellet volume determined? [Section 13.2.1]	Requirement			
5.2.8	Is there a set of standards for comparison of pellet size?	Recommendation GLP			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
5.2.9 Are SOPs for Envirochek® and Filta-Max® filter sample centrifugation available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
5.2.10 Other than issues noted for items 5.2.1 through 5.2.9 (if any) was Envirochek® or Filta-Max® filter sample centrifugation demonstrated successfully?				
6 Purification and Slide Preparation		**Technician:**		
6.1 Is an approved IMS kit/manufacturer used?	Method Procedure GLP			
6.2 Is the supernatant from the centrifuged sample aspirated no lower than 5 mL above the pellet or no lower than 8 mL (Filta-Max xpress®)? [Section 13.2.2 or manufacturer's instructions]	Requirement			
6.2.1 Are the samples aspirated using the pipette, with the documented internal diameter, as specified in the SOP?	Critical			
6.2.2 Is the proper rate (mL/min) or pressure (psi) maintained throughout aspiration?	Method Procedure			
6.2.3 For Filta-Max xpress®, is 8-mL volume marked on the 500-mL centrifuge tube per manufacturer's instructions?	Critical			
6.3 Is the pellet vortexed a sufficient time for resuspension? [Section 13.2.3]	Method Procedure			
6.4 Is the resuspended pellet volume quantitatively transferred to the flat-sided tube (2 rinses)? [Section 13.3.2.1]	Method Procedure			
6.5 Are the IMS beads thoroughly resuspended prior to addition to the flat-sided tube? [Section 13.3.2.2]	Method Procedure			
6.6 Is the flat-sided tube rotated at 18 rpm for 1 hour at room temperature? [Section 13.3.2.6]	Method Procedure			
6.7 Is the rotator speed checked and calibrated annually?	Critical GLP			
6.8 Is flat-sided tube correctly placed in magnet and rocked through 90 degrees about once per second? [Section 13.3.2.9]	Method Procedure			
6.9 Is all the liquid removed when decanting is performed with the magnet up? [Section 13.3.2.11]	Method Procedure			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
6.10 Is the sample quantitatively transferred from the flat-sided tube to the microcentrifuge tube (2 rinses)? [Section 13.3.2.13]	Method Procedure			
6.11 Are extra rinses performed appropriately when needed? The laboratory rinses: (A) IMS beads in the flat-sided tube prior to transfer (B) flat-sided tube, not IMS beads, prior to transfer (C) IMS beads in microcentrifuge tube prior to dissociation.		Circle one: A B C		
6.12 Is standard NaOH (5 µL, 1N) and standard HCl (50 µL, 0.1N) used? [See note on pg 49]	Requirement GLP			
6.13 Is sample vortexed vigorously for 50 seconds immediately after the addition of acid and 30 seconds after the sample has set for 10 minutes at room temperature? [Section 13.3.3]	Method Procedure			
6.14 Is a second dissociation performed? [Section 13.3.3.10]	Requirement			
6.15 When the second dissociation is performed, does the laboratory: (A) use a second slide (B) add the additional volume to the original slide?		Circle one: A B		
6.16 Are the slides clearly labeled so they can be associated with the correct sample? [Section 13.3.3.7]	Requirement			
6.17 What type of slides is used?	GLP			
6.18 Is slide dried at: (A) room temperature, (B) 35º to 42ºC, or (C) in the refrigerator? [Section 13.3.3.12]		Circle one: A B C		
6.19 If the slide is warmed, is incubator or slide tray calibrated and labeled?	Critical GLP			
6.20 Are SOPs available in the work area for sample purification and slide preparation, and does laboratory practice reflect written procedures?	Critical GLP			
6.21 Other than issues noted for items 6.1 through 6.20 (if any) was purification and slide preparation demonstrated successfully?				
7 Sample Staining		**Technician:**		
7.1 What staining kit/manufacturer is used? [Section 14.2]	GLP			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
7.2 Is FITC stain applied according to manufacturer's directions? [Section 14.2]	Method Procedure			
7.3 Are positive and negative staining controls performed? [Section 14.1]	Requirement			
7.4 Are the slides incubated in a humid chamber in the dark at room temperature for approximately 30 minutes or per manufacturer's directions? [Section 14.3]	Method Procedure			
7.5 Are the labeling reagents rinsed away properly after incubation, without disturbing the sample? [Section 14.5]	Method Procedure			
7.6 Was the working DAPI stain prepared the day it was used? [Section 7.7.2]	Method Procedure			
7.7 Is stock DAPI stored at 1 to 10°C in the dark? [Section 7.7.1]	Method Procedure			
7.8 Is the DAPI stain applied properly and allowed to stand for a minimum of 1 minute? [Section 14.6]	Method Procedure			
7.9 Is the DAPI stain rinsed away properly without disturbing the sample? [Section 14.7]	Method Procedure			
7.10 Is the mounting media applied properly?	Method Procedure			
7.10.1 What type of mounting media is used?	GLP			
7.10.2 Are all the edges of the cover slip sealed well with clear fingernail polish, unless Elvenol is used? [Section 14.9]	Method Procedure			
7.11 Are the finished slides stored in a humid chamber in the dark at 1 to 10°C (humid chamber not required for Evenol)? [Section 14.10]	Method Procedure			
7.12 Are SOPs for sample staining available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
7.13 Other than issues noted for items 7.1 through 7.12 (if any) was sample staining demonstrated successfully?				
8 Microscope and Examination				
8.1 Is microscope equipped with appropriate excitation and band pass filters for examining FITC labeled specimens as demonstrated with lab, and auditor provided, positive staining control? [Section 6.9.2]	Requirement GLP			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
8.2 Is microscope equipped with appropriate excitation and band pass filters for examining DAPI labeled specimens as demonstrated with lab, and auditor provided, positive staining control? [Section 6.9.3]	Requirement GLP			
8.3 Does the microscope have appropriate objectives and filters for HMO or DIC, which change easily to and from epifluorescence? [Section 6.9.1]	Requirement GLP			
8.4 Are all portions of the microscope, from the light sources to the oculars, properly adjusted? [Section 10.3.1.1]	Requirement			
8.5 Is microscope completely cleaned every week? [Section 10.4]	Requirement GLP			
8.6 Does the microscope have a 20X scanning objective? [Section 6.9.1]	Requirement GLP			
8.7 Does the microscope have a 100X oil immersion objective? [Section 6.9.1]	Requirement GLP			
8.8 Is the microscope equipped with an ocular micrometer? [Section 6.9.1]	Requirement GLP			
8.9 Is a stage micrometer available to laboratory? [Section 10.3.5]	Requirement			
8.10 Is a calibration table for 100X objective located close to the microscope(s)? [Section 10.3.5]	Requirement			
8.11 Has the mercury bulb been used less than the maximum hours recommended by the manufacturer? [Section 10.3.2.11]	Recommendation			
8.12 Does the laboratory have a preventative maintenance agreement in place to service the microscope annually?	Critical GLP			
8.13 Are SOPs for sample examination available in the work area, and does laboratory practice reflect written procedures?	Critical GLP			
8.14 Other than issues noted for items 8.1 through 8.13 (if any) was Microscope and Examination demonstrated successfully?				
9 Positive Staining Control and OPR Slides				
9.1 Does the positive staining control slide sent by the laboratory contain *Cryptosporidium* oocysts at the appropriate fluorescence intensity for FITC? [Section 15.2.1.3]	Requirement			

Item to be evaluated	Classification	Yes, No, NA or Unknown	Comments	Response Requested
9.2 Does the positive staining control slide sent by the laboratory contain *Cryptosporidium* oocysts at the appropriate fluorescence intensity for DAPI? [Section 15.2.1.3]	Requirement			
9.3 Does the positive staining control slide sent by the laboratory contain an appropriate level of background fluorescence?	Recommendation			
9.4 Is concentration of oocysts on the positive staining control slide appropriate? [Section 14.1.1]	Requirement			
9.5 Does the OPR slide sent by the laboratory contain *Cryptosporidium* oocysts at the appropriate fluorescence intensity for FITC? [Section 15.2.2]	Requirement			
9.6 Does the OPR slide sent by the laboratory contain *Cryptosporidium* oocysts at the appropriate fluorescence intensity for DAPI? [Section 15.2.2]	Recommendation			
9.7 Does the OPR slide sent by the laboratory contain an appropriate level of background fluorescence?	Requirement			
9.8 Does the technical auditor's count of *Cryptosporidium* oocysts and *Giardia* cysts on the OPR slide sent by the laboratory agree within 10% of laboratory count?	Requirement			

10 Onsite Sample Processing			
Method Step	**Name**	**Position**	**Demonstrated Technique Successfully yes/no**
Spiking – *(filter type)*			
Filtration - *(filter type)*			
Spiking flat-sided tube, and processing IMS control			
Aspiration and transfer from 250 mL bottle			

11 Onsite Blind Spike Results						
Sample	**Crypto Spike Value**	**Crypto Count**	**Crypto Recovery (%)**	***Giardia* Spike Value**	***Giardia* Count**	***Giardia* Recovery (%)**

12 Evaluation of Blind Spike Results – Comments and Recommendations		
Classifications	**Comments**	**Response Requested**

13 Was analyst microscope operation acceptable? (yes/no)				
Classification	**Requirement**	**Requirement [Section 10.3.4]**	**Requirement [Section 10.3.4.2]**	**Requirement [Section 10.3.6]**
Name	**Position**	**Adjust Interpupillary Distance**	**Focus both eyepieces**	**Establish Kohler Illumination**

14 Slide Count and Analyst Verification Results (yes/no)

Analyst	Crypto Count Within 10% of Target Count	*Giardia* Count Within 10% of Target Count	Examine and Record Characteristics	Measurement (100X)	Demonstrated Internal Structures

15 Evaluation of Analyst Microscopy and Examination Skills – Comments and Recommendations		
Classifications	**Comments**	**Response Requested**

APPENDIX H

Rubrics for Verification of *Cryptosporidium* Laboratory QA

Verification of *Cryptosporidium* Laboratory Quality Assurance Technical Evaluation

Laboratory Name: **Date:**

Technical Auditor:

		1	2	3	4	Score
Checklist C Technical Review	**Section 1**					
	1.1 - 1.4	Neither safety procedures nor bench space are appropriate	Bench space is adequate; safety procedures are not appropriate	Safety procedures are appropriate; bench space is not adequate	Safety procedures and bench space are appropriate	
	Section 2					
	2.1 - 2.3	Reagents are not properly labeled; SOPs are not followed	Reagents are properly labeled; SOPs are not followed	Reagents are not properly labeled; SOPs are followed	Reagents are properly labeled and SOPs are followed	
	Section 3					
	3.1 - 3.5	Sample spiking was not demonstrated successfully	Critical items not met; multiple recommendations	Critical items met; ≤ 1 recommendation	Sample spiking was demonstrated successfully without recommendations; critical items met	
	Section 4					
	4.1	Envirochek™ filtration was not demonstrated successfully	<2 Required items met; multiple recommendations	3 or 4 Required items met; few recommendations	Envirochek™ filtration was demonstrated successfully without recommendations; all required and critical items met	
	4.2	Envirochek™ elution was not demonstrated successfully	Critical item not met; >3 recommendations	Critical item met; ≤ 3 recommendations	Envirochek™ elution was demonstrated successfully without recommendations	
	4.3	Filta-Max™ filtration was not demonstrated successfully	<2 Required items met; multiple recommendations	3 or 4 Required items met; few recommendations	Filta-Max™ filtration was demonstrated successfully without recommendations	NA

	4.4	Filta-Max™ Wash station elution was not demonstrated successfully	1 Required item met; multiple recommendations	2 or 3 Required items met; few recommendations	Filta-Max™ Wash station elution was demonstrated successfully without recommendations	
Checklist C Technical Review	**4.5**	Filta-Max™ stomacher elution was not demonstrated successfully	Required item not met; multiple recommendations	Required item met; few recommendations	Filta-Max™ stomacher elution was demonstrated successfully without recommendations	
	4.6	Filta-Max xpress™ elution was not demonstrated successfully	2 Required items not met; multiple recommendations	2 Required items met; few recommendations	Filta-Max xpress™ elution was demonstrated successfully without recommendations	
	Section 5					
	5.1	Filta-Max™ concentration was not successfully demonstrated	2 Required items not met; multiple recommendations	2 Required items met; few recommendations	Filta-Max™ concentration was successfully demonstrated without recommendations	
	5.2	Centrifugation was not demonstrated successfully	Required item not met; Critical items not met; multiple recommendations	Required item met; Critical items met; few recommendations	Centrifugation was demonstrated successfully without recommendations	
	Sections 6, 7, 8					
	6.1 - 6.21	Purification and Slide preparation were not demonstrated successfully	Required items not met; Critical items not met; multiple recommendations	Required items met; Critical items met; few recommendations	Purification and Slide preparation were demonstrated successfully without recommendations	
	7.1 - 7.13	Sample staining was not demonstrated successfully	Required item not met; Critical item not met; multiple recommendations	Required item met; Critical item met; few recommendations	Sample staining was demonstrated successfully without recommendations	
	8.1 - 8.14	Microscope is not properly equipped; examination unsuccessful	Microscope is properly equipped; FITC and/or DAPI examination unsuccessful	Microscope is properly equipped; FITC and DAPI examination was successful with recommendations	Microscope is properly equipped; examination was successful without recommendations	
	Section 9					

Checklist C Technical Review	**PSC/OPR Slides**	**Score from PSC/OPR Evaluation = %**				
	Section 10					
	Aspiration	Aspiration did not follow laboratory SOP	N/A	Aspiration did follow laboratory SOP with recommendations	Aspiration did follow laboratory SOP without recommendations	
	Transfer to flat-sided tube	Transfer did not follow laboratory SOP	N/A	Transfer did follow laboratory SOP with recommendations	Transfer did follow laboratory SOP without recommendations	
	Section 11 *Cryptosporidium* Results					
	Spiking and Filtration Sample with Matrix Filter - HV	Method criteria not met	Method criteria met; recovery is lower than laboratory's results from previous PT round and overall average	Method criteria met; recovery is higher than laboratory's results from previous PT round and lower than overall average	Method criteria met; recovery is higher than laboratory's results from previous PT round and overall average	
	Spiking and Filtration Sample with Matrix Filter - FM	Method criteria not met	Method criteria met; recovery is lower than laboratory's results from previous PT round and overall average	Method criteria met; recovery is higher than laboratory's results from previous PT round and lower than overall average	Method criteria met; recovery is higher than laboratory's results from previous PT round and overall average	
	IMS Control Sample with Matrix	Recovery ≤ 20%	Recovery >20% and ≤ 40%	Recovery >40% and ≤ 60%	Recovery >60%	
	Section 13					
	Microscope Adjustment (applies to analysts not evaluated during previous audit)	Met none of the microscope adjustment requirements	Met ocular adjustment requirements; did not meet Kohler requirement	Met Kohler requirement; did not meet ocular adjustment requirements	Met all 3 microscope adjustment requirements	
Checklist C Technical Review	**Section 14**					
	Slide Counts (circle one response)	**PASS FAIL**				

	Analyst Verification	**Score for Analyst Verification Evaluation = %**				
	Good Laboratory Practice (GLP) summary	Met none of the GLP items	Met <XX of the GLP items	Met >XX of the GLP items	Met all GLP items with no recommendations	
					Total	0
					Total Possible Score	52
					Normalized Score (%)	0%

Verification of *Cryptosporidium* Laboratory QA Positive Staining Control and OPR Evaluation

Laboratory Name: **Date:**

Technical Auditor: **Organism/Slide ID:**

		1	2	3	4	Score
Positive Stain Control	**Number of Organisms**	organisms in very low numbers (<~25 oo/cysts)	organisms in low numbers (~25 to ~100 oo/cysts)	organisms in relatively high numbers(~100 to <~200 oo/cysts)	organisms in high numbers (~200 to <~400 oo/cysts)	
	FITC Fluorescence*	consistently weak (1+); inadequate; not crisp	inconsistent; more weak (1+) than strong (3+); less than adequate; more not crisp than crisp	inconsistent; more strong (3+) than weak (1+); adequate; more crisp than not crisp	consistently strong (3+); superior; crisp	
	DAPI Fluorescence**	DAPI negative in majority of oocysts; nuclei stained consistently weak (1+); inadequate	distinct nuclei in less than half of the oocysts; nuclei stain inconsistent; more weak (1+) than strong (3+); less than adequate	distinct nuclei stained in majority of oocysts; nuclei stain inconsistent; more strong (3+) than weak (1+); adequate	distinct nuclei stained in majority of oocysts; nuclei stain consistently strong (3+); superior	
	Background Fluorescence	excessive, interfering	distracting	exists but does not interfere	minimal to nonexistent	
OPR	**Number of Organisms**	difference in number counted >20%	16-20% difference	10-15 % difference	difference in number counted <10%	
	FITC Fluorescence*	consistently weak (1+); inadequate; not crisp	inconsistent; more weak (1+) than strong (3+); less than adequate; more not crisp than crisp	inconsistent; more strong (3+) than weak (1+); adequate; more crisp than not crisp	consistently strong (3+); superior; crisp	
	DAPI Fluorescence**	DAPI negative in majority of oocysts; nuclei stained consistently weak (1+), inadequate	distinct nuclei in less than half of the oocysts; nuclei stain inconsistent; more weak (1+) than strong (3+); less than adequate	distinct nuclei stained in majority of oocysts; nuclei stain inconsistent; more strong (3+) than weak (1+); adequate	distinct nuclei stained in majority of oocysts; nuclei stain consistently strong (3+), superior	

	Background Fluorescence	excessive, interfering	distracting	exists but does not interfere	minimal to nonexistent	
*FITC Fluorescence: brilliant apple-green ovoid or spherical objects with brightly highlighted edges; compared for all organisms over complete well slide at 200x **DAPI Fluorescence: light blue internal staining (no distinct nuclei) with green rim, intense internal blue stain, or distinct nuclei; compared for all organisms over complete well slide at minimum of 400x Fluorescence intensity scale: 1+ = weak, 2+=medium, 3+=strong; for comparison, see FITC and DAPI staining examples in the LT2 On-line Microscopy Training Module (http://www.epa.gov/safewater/lt2/training/index.html#)					**Total**	0
Comments: PSC:					**Total Possible Score**	32
OPR:					**Normalized Score (%)**	0
Recommendations & Commendations:						

Laboratory Name:					**Date:**	
Analyst Name(s):						
Technical Auditor:						
Characterizations		**1**	**2**	**3**	**4**	**Score**
DAPI	**Compare analyst's and auditor's independent characterization of 3 oocysts**	No agreement*	Agreement* for 1 oocyst	Agreement* for 2 oocysts	Agreement* for 3 oocysts	
DIC						
DIC	**Demonstrated Internal Structures**	No	NA	NA	Yes	
	Measurement with 1000x objective	No	NA	NA	Yes	
Examine Slides <4 hours per day		No	NA	NA	Yes	
					Total per analyst	
					Maximum score possible	16
					Total Possible Score for Laboratory (# analysts * 20)	
					Normalized Score for Laboratory (%)	

*Agreement is defined as the auditor accepting the characterization by the analyst based on the analyst's explanation. For example, analyst characterizes the oocyst with 2 DAPI-stained nuclei and auditor asks for explanation. During the explanation, the analyst focuses the specimen and adjusts contrast and resolution such that 2 DAPI-stained nuclei are observable; this counts as one characterization agreement.

Photographs used:	*Cryptosporidium*: *Giardia*:
Comments: **Recommendations:**	
Commendations:	

APPENDIX I

Verification of *Cryptosporidium* Laboratory QA
Analyst Verification Characterization Form

Analyst Verification Characterizations

Laboratory:		
Analyst's Name:	**Analyst's Position:**	**QA Manager's Name:**
Analyst's Signature* and Date:		
QA Manager's Signature* and Date:		

**By signing this form the analyst and QA Manager are certifying that the characterizations were performed independently by the above signed analyst.*

Measurement and Characterization of Three *Cryptosporidium* Oocysts

Source of Slide:			DAPI -	DAPI +		D.I.C.		
# of Object located by FA	Shape (oval or round)	Size L x W (µm)	Light blue internal staining, no distinct nuclei, green rim	Intense blue internal staining	Number of nuclei stained sky blue	Empty oocysts	Oocysts with amorphous structure	Oocysts with internal structure Number of sporozoites
1								
2								
3								

Measurement and Characterization of Three *Giardia* Cysts

Source of slide:			DAPI -	DAPI +		D.I.C.				
# of Object located by FA	Shape (oval or round)	Size L x W (µm)	Light blue internal staining, no distinct nuclei, green rim	Intense blue internal staining	Number of nuclei stained sky blue	Empty cysts	Cysts with amorphous structure	Cysts with internal structure		
								Number of nuclei	Median body	Axonemes
1										
2										
3										

For Official Use Only
Technical Auditor's Name:
Technical Auditor's Signature and Date:

Comments:

APPENDIX J

Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* Under the Long Term 2 Enhanced Surface Water Treatment Rule

Clarification of Basis and Procedures for Downgrading/Suspending Approval for Laboratories for the Analysis of *Cryptosporidium* Under the Long Term 2 Enhanced Surface Water Treatment Rule

The U.S. Environmental Protection Agency's (EPA's) Office of Ground Water and Drinking Water, in the Office of Water, prepared this document. EPA intends to use this document for its own use in approving laboratories for analysis of drinking water contaminants. In order to assume primary enforcement responsibility for the drinking water regulations, a State must either have available laboratory facilities, approved by the Administrator, capable of conducting analytical measurements of drinking water contaminants, or establish and maintain its own program for approval of laboratories. States wishing to adapt the procedures and criteria of this document for their own approval program should revise it to accurately reflect their State approval program.

This is a protocol that is intended to clarify EPA's intended practice and procedures for laboratory approval and to reflect good laboratory practice and standard proficiency evaluation in the industry; it is not a regulation. While EPA intends generally to follow the procedures laid out in this document, not every situation is reflected in these procedures and EPA may need to address case-specific situations in ways that differ from the procedures spelled out here. This document is not a rule, is not legally enforceable, and does not confer legal rights or impose legal requirements upon anyone. EPA welcomes comment on this protocol and may decide to revise this document without public notice to reflect changes to its approach or to clarify and update the text.

- **"*Approved Laboratories*" have demonstrated, and continue to demonstrate, proficient and reliable detection and enumeration of *Cryptosporidium* in surface water sources for public water systems. They have passed all elements in the Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Lab QA Program) and continue to successfully participate in all program activities. Approved Laboratories notify the Approval Authority (EPA individual(s) administering the program or State individual(s) administering an equivalent laboratory certification program) of loss of key personnel or essential equipment, change in policies or procedures that directly affect the validity of data, and any other change affecting the capability of the laboratory, including change in location.**
- **"*Provisionally Approved Laboratories*" have deficiencies, but demonstrate their ability to consistently produce data of known quality. They continue to successfully participate in all Lab QA Program activities. A Provisionally Approved Laboratory may analyze drinking water samples for Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) compliance purposes if the laboratory has identified themselves as Provisionally Approved to their clients and any reports clearly state that the laboratory's status is "Provisionally Approved."**
- **"*Not Approved*" designates a laboratory that has either not participated in the Lab QA Program, or has applied to the program, but possesses deficiencies and, in the**

opinion of the Approval Authority, does not consistently produce data that have met all applicable method quality control (QC) requirements or has falsified data.

Basis for Downgrading to "Provisionally Approved" Status

An Approved Laboratory (referred to as "laboratory") may be downgraded to "Provisionally Approved" status for *Cryptosporidium* for any of the following reasons:

- Failure to analyze samples for the LT2ESWTR according to the December 2005 version of EPA Method 1623 or EPA Method 1622, including all QA/QC criteria;
- Failure to document a minimum of 22 percent for on-going precision and recovery (OPR) values in an updated QC chart prior to analysis of LT2ESWTR samples at the frequency required in Section 9.7 of the method;
- Failure to demonstrate proficiency based upon acceptable matrix spike recoveries for all modifications of the method procedures per Section 9.1.2 of the method;
- Failure to submit valid Proficiency Test (PT) results or meet PT acceptance limits described by the Approval Authority for the first two initial testing events, or two out of three regular testing events administered by a vendor authorized by the Approval Authority.
    - Acceptance Limits: laboratory mean recovery between ±2 standard deviations (SD) of the mean recovery for all approved laboratories in a given test event.
        - Recoveries below the mean recovery minus 2 SD will fail the PT test event.
        - Recoveries higher than the mean recovery plus 2 SD trigger additional evaluation, which may include one or more of the following:
            - On-site evaluation
            - Presence of a proctor when processing PT samples during the next test event
            - Submission of PT microscope slides to the Approval Authority before the expiration of holding time during the next test event;
- Failure to submit PT slides within three weeks of PT test event when requested by the Approval Authority;
- Failure to maintain records of method modifications per Section 9.1.2.2 of the method;
- Failure to notify the Approval Authority of loss of key personnel or essential equipment, change in policies or procedures that directly affect the validity of data, or other changes affecting the capability of the laboratory, including change in location. Laboratory approval does not automatically survive such changes; the Approval Authority may request an on-site or off-site evaluation and/or further proof of compliance with all applicable method requirements;
- Failure to submit on-site evaluation materials and any other requested information within the time period requested by the Approval Authority;
- Failure to participate satisfactorily in the Approval Authority Lab QA Program and demonstrate proficiency based upon:
    - Sample and method holding time records;
    - Analyst verification skills;
    - Relative quality of positive staining control and OPR slides;

- Acceptable performance of QC checks, including but not limited to, blind slide counts; and
- Acceptable precision and recovery values for all method variations.

Procedures for Downgrading to "Provisionally Approved" Status

- The Approval Authority will notify the laboratory director or owner of its intent to downgrade after becoming aware of the situation warranting downgrading;
- The laboratory director should review the problems cited, and within 30 days of receipt of the letter, send a letter to the Approval Authority specifying immediate corrective actions that are being taken;
- The Approval Authority will consider the adequacy of the response and notify the laboratory in writing of its approval status, generally within 14 days of receipt of the laboratory's response;
- After the Approval Authority notifies a laboratory, the Approval Authority will post the laboratory's status on the Web site list of laboratories and may schedule an on-site evaluation of the laboratory;
- The laboratory should identify and correct its problem(s) to the Approval Authority's satisfaction within 30 days of being notified of the downgrade or have approval status suspended;
- A Provisionally Approved laboratory may continue to analyze samples for compliance purposes, but must identify its status as Provisionally Approved on any report;
- A laboratory may request that the Approval Authority or State provide technical assistance to help identify and resolve any problem; however, adequate performance is the laboratory's responsibility and Approval Authority assistance should not delay the downgrading procedure.

Basis for Suspending Approval Status

A laboratory may be downgraded from Approved or Provisionally Approved status to "Not Approved" for any of the following reasons:

- Repeated verification that all applicable method QC requirements have been followed, when in fact they have not all been met;
- Repeated failure to document acceptable OPR values prior to analysis of LT2ESWTR samples;
- Reporting PT data from another laboratory as its own;
- Falsification of data or other deceptive practices, including false verification that data submitted to the Data Collection and Tracking System (DCTS) were generated using approved methods and met all method QA/QC criteria;
- Refusal or failure to participate in on-site or off-site evaluations conducted by the Approval Authority.

Basis for Suspending Provisionally Approved Status

- Failure to provide a letter to the Approval Authority within 30 days that adequately explains what immediate corrective actions were taken;
- Failure to identify and correct problems in response to downgrade within 30 days;
- Failure to provide accurate OPR control charts to the Approval Authority;

- Failure to submit valid PT results for the next two consecutive authorized PT test events within the acceptance limits specified;
- Continued failure to use the analytical methodology specified in the regulations;
- Failure to correct deviations identified during an on-site evaluation within 30 days;
- Failure to provide requested demonstration, materials and documentation within 30 days, including:
    - Acceptable matrix spike recoveries for all method variations per Section 9.1.2 of the method;
    - Bench sheets, examination forms or OPR charts for any samples requested;
    - Remote analyst verification;
    - Recent positive staining control and OPR microscope slides, one of each;
    - Blind slide counts for each analyst.

Procedures for Suspension

The Approval Authority will notify the laboratory, in writing, of its intent to suspend approval. If the laboratory wishes to request reconsideration of this decision, it should submit such a request in writing to the Approval Authority within 30 days of receipt of the notice of intent to suspend approval. The laboratory will generally be downgraded immediately to "Provisional Approval" in the interim while the suspension is being considered. If no request for reconsideration is filed, approval will be suspended.

The request for reconsideration should be supported with an explanation of the reasons for the challenge and should be signed by a responsible official from the laboratory, such as the president/owner for a commercial laboratory, the laboratory supervisor of a municipal laboratory, or the laboratory director for a State or Regional laboratory.

The Approval Authority will make a decision and notify the laboratory in writing, generally within 30 days of receipt of the request for reconsideration. If the request is determined to be valid, the Approval Authority will take appropriate measures to reevaluate the facility and notify the laboratory, in writing, of its decision, generally within 60 days of the reevaluation.

Denial of the request will generally result in suspension of the laboratory's approval. Once approval is suspended, a public water system may not use the laboratory to analyze source water samples for compliance with LT2ESWTR source water monitoring requirements. The laboratory should notify its clients that it is no longer approved and will not accept any more LT2ESWTR samples for analysis.

Upgrading or Reinstatement of Approval

Subject to the availability of resources, the Approval Authority will consider written requests from the laboratory to seek upgrading or reinstatement of approval. Requests should state the reasons why the laboratory should regain its approval status. The laboratory should demonstrate that all deficiencies have been corrected and successfully complete two consecutive authorized PT test events within acceptance limits for Provisionally Approved laboratories or three consecutive authorized PT test events within acceptance limits for suspended laboratories. The authorized PT test events being described here are those submitted to all laboratories in the Lab QA Program, not special issue blind samples purchased independently from the vendor. The

laboratory should provide evidence why the reasons for downgrading or suspension are no longer applicable and explain its technical competence. Acceptable demonstration of technical competence may include an on-site evaluation and/or any other measure the Approval Authority deems appropriate. The Approval Authority will consider compliance history, corrective actions implemented by the laboratory, effectiveness of corrective actions, and professional judgment of the Approval Authority.

Grievances

Laboratories with grievances during the authorized PT events or regarding participation in the Lab QA Program should immediately contact the Program Manager at the Approving Authority and try to remedy the problem. When the laboratory feels they have not gotten immediate or satisfactory results, they should contact the supervisor at the Approving Authority. The management at the Approving Authority will work with the Program Manager to quickly address grievances. A final decision for all grievances will be made generally within 30 days of contacting the Approving Authority.

APPENDIX K

Updated Procedures for Evaluating Ongoing Precision and Recovery Quality Control

Updated Procedures for Evaluating Ongoing Precision and Recovery Quality Control Calculated from February and May 2007 Proficiency Testing Data
February 23, 2009

Introduction

The procedures for evaluating Ongoing Precision and Recovery (OPR) results used by the "Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act," hereafter referred to as the "Lab QA Program," were originally based on data collected during the Information Collection Rule Supplemental Surveys from March 1999 to February 2000. Table 1 summarizes the data set used to develop a criterion for evaluation. The OPR mean recovery was calculated from data performed by a limited number of laboratories, using the earliest versions of Method 1623 and Method 1622 (1999), and a filter that is no longer used by any of the laboratories currently participating in the Lab QA Program. Therefore, EPA expects that the minimum recovery criterion developed using this old data underestimates current method and laboratory capability.

To develop a more appropriate criterion, updated limits for lower recovery values were calculated using the data from the February and May 2007 proficiency test (PT) rounds. The PT samples in these two studies served as good surrogates for OPR samples, because they involved spiking *Cryptosporidium* oocysts into reagent water rather than a matrix. The data set is summarized in Table 1. This updated minimum criterion of 22% recovery provides a better assessment of routine laboratory performance than the original value for the following reasons: 1) the data set is more current and is based on more samples (a total of 333); 2) 52 more laboratories are included in the data set; 3) data were generated using the 2005 version of Method 1623, which is the required version for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR); 4) data were generated using filters currently used to analyze LT2ESWTR samples rather than those filters used originally; and 5) the number of oocysts spiked into the samples was unknown to the laboratories.

Table 1. Summary of Original and Updated OPR Values

	Original Value	Updated Value
Criterion	11%	22%
Data Set	ICRSS data generated in 1999 and 2000	PT data generated during the February and May 2007 rounds
Number of Laboratories	6	58
Number of Samples	293	333
Method Version Used	1999 version of Method 1623 and Method 1622	2005 version of Method 1623
Filter currently in use	No	yes

Blind vs. Unblind	Unblind	Blind
Statistical analysis	Estimation of variance within & between labs	Estimation of variance within & between labs

Details of the Calculation of OPR Minimum Recovery Criterion

To calculate the OPR recovery criterion, estimates of variance attributable to four different sources were calculated: variability between laboratories, variability between PT rounds, variability between laboratory-and-round (i.e., attributable to an interaction between round and laboratory), and variability within round and laboratory (i.e., analytical variability). There were a few laboratories that performed PT analyses using multiple filters. For the purpose of these calculations, the two sets of analyses were two separate entities; in other words, the two lab/filter combinations were treated as two different laboratories. The different variance components were calculated using PROC MIXED from SAS version 8 using the maximum likelihood method of estimation on recovery data. Details on the maximum likelihood estimation can be found in SAS/STAT User's Guide.[1]

Estimates of between laboratory variance, between round variance, between laboratory-and-round variance, and within laboratory-and-round variance were labeled s^2_L, s^2_R, s^2_{LR} and s^2_w, respectively. The combined standard deviation (s_c) is:

$$s_c = \sqrt{(1+\frac{1}{\overline{L}})s_L^2 + (1+\frac{1}{\overline{R}})s_R^2 + (1+\frac{1}{\overline{L}*\overline{R}})s_{LR}^2 + (1+\frac{1}{\overline{L}*\overline{R}*n})s_w^2}$$

Where:

$\overline{L} = \frac{C}{R}$ = *average number of laboratory/filter combinations per round*

$\overline{R} = \frac{C}{L}$ = *average number of rounds per laboratory/filter combination*

n = *number of replicates per laboratory/filter and round (3)*

C = *total number of laboratories/filters/rounds*

n_T = *total number of replicates over all labs/filters/rounds*

Upper and lower recovery limits for OPR samples were then calculated as:

$$X_{Mean} \pm t_{(0.975,df)} * s_c$$

Where:

X_{mean} *= the mean recovery of all samples*

df is calculated using Satterthwaite's estimate as given below:

$$df = \frac{s_c^4}{\frac{((1+\frac{1}{\overline{L}})s_L^2)^2}{\overline{L}-1} + \frac{((1+\frac{1}{\overline{R}})s_R^2)^2}{\overline{R}-1} + \frac{((1+\frac{1}{\overline{L}*\overline{R}})s_{LR}^2)^2}{(\overline{L}-1)(\overline{R}-1)} + \frac{((1+\frac{1}{\overline{L}*\overline{R}*n})s_w^2)^2}{\overline{L}*\overline{R}*(n-1)}}$$

Comparison of OPR Minimum Recovery Limit to PT OPRs

OPR data that were submitted in conjunction with *Cryptosporidium* PT samples were compared

1 SAS Institute Inc. 1994. SAS/STAT User's Guide, Volume 2, GLM-VARCOMP. Version 6, 4th Edition, June 1994.

to the newly calculated OPR minimum recovery limit. When applying the minimum percent recovery criterion of 22% to the 1125 OPR samples submitted between September 2002 and October 2008, 41 samples (3.6%) did not meet the criterion. This comparison validates the use of the new criterion, because it demonstrates that laboratories can routinely meet the 22% recovery criterion.

APPENDIX L

Burden Tables

Table 1. Participating Laboratories Seeking Continued Approval

Activity	Legal $65.00/hour	Management $72.00/hour	Technical $54.00/hour	Clerical $41.00/hour	Respondent Hours	Labor Costs	Capital/Startup	O&M Costs	Number of respondents/year	Total hours/year	Total cost/year	Labor Cost/ Activity	O&M Cost/Activity	Labor Cost/Hr
Complete and submit re-audit package	0	3	12	1	16				21	336				
	$ -	$ 216.00	$ 648.00	$ 41.00		$ 905.00	$ -	$15.00			$ 19,320.00	$19,005.00	$ 315.00	$ 56.56
Perform off-site re-evaluation activities	0	2	14	1	17				21	357				
	$ -	$ 144.00	$ 756.00	$ 41.00		$ 941.00	$ -	$350.00			$ 27,111.00	$19,761.00	$ 7,350.00	$ 55.35
Host on-site re-evaluation	0	9	20	2	31				21	651				
	$ -	$ 648.00	$ 1,080.00	$ 82.00		$ 1,810.00	$ -	$500.00			$ 48,510.00	$38,010.00	$ 10,500.00	$ 58.39
Perform and report 3 sets of ongoing performance tests (OPT)	0	5.8	35.3	4.4	45.5				63	2867				
	$ -	$ 417.60	$ 1,906.20	$ 180.40		$ 2,504.20	$ -	$1,692.00			$ 264,360.60	$157,764.60	$ 106,596.00	$ 55.04
Perform follow-up action for poor PT results	0	3	20	1	24				9	216				
	$ -	$ 216.00	$ 1,080.00	$ 41.00		$ 1,337.00	$ -	$564.00			$ 17,109.00	$12,033.00	$ 5,076.00	$ 55.71
Perform and report 3 sets of OPTs for each additional method version	0	5.8	35.3	4.4	45.5				4	182				
	$ -	$ 417.60	$ 1,906.20	$ 180.40		$ 2,504.20	$ -	$1,692.00			$ 16,784.80	$10,016.80	$ 6,768.00	$ 55.04
									Total	4,609	$ 393,195.40	$256,590.40	$ 136,605.00	

O&M Costs to Perform off-site re-evaluation activities: cost of slides
O&M Costs to Host on-site re-evaluation: cost of spikes and demonstration samples
O&M Costs to Perform and report 3 sets of ongoing performance tests (OPT): $188/sample X 3 OPT samples/set X 3 sets per year
O&M Costs to Perform follow-up action for poor PT results: $188/sample X 3 OPT samples/set per year
O&M Costs to Perform and report 3 sets of OPTs for each additional method version: $188/sample X 3 OPT samples/set X 3 sets per year
Number of respondents/year to Complete and submit re-audit package: Total of approximately 63 laboratories seeking approval divided by three years
Number of respondents/year to Perform off-site re-evaluation activities: Total of approximately 63 laboratories seeking approval divided by three years
Number of respondents/year to Host on-site re-evaluation: Total of approximately 63 laboratories seeking approval divided by three years
Number of respondents/year to Perform and report 3 sets of ongoing performance tests (OPT): Approximately 63 labs participating in the Lab QA Program
Number of respondents/year to Perform follow-up action for poor PT results : Estimated number of laboratories that will have to perform follow-up due to poor PT results
Number of respondents/year to Perform and report 3 sets of OPTs for each additional method version: Number of labs performing more than one method version

Table 2. Laboratories Seeking Initial Approval

Activity	Legal $65.00/hour	Management $72.00/hour	Technical $54.00/hour	Clerical $41.00/hour	Respondent Hours	Labor Costs	Capital/Startup	O&M Costs	Number of respondents/year	Total hours/year	Total cost/year	Labor Cost/ Activity	O&M Cost/Activity	Labor Cost/Hr
Complete and submit application	0	3	12	1	16				2	32				
	$ -	$ 216.00	$ 648.00	$ 41.00		$ 905.00	$ -	$ 15.00			$ 1,840.00	$ 1,810.00	30.00	$ 56.56
Perform and report initial performance tests (IPT)	0	2	32	2	36				2	72				
	$ -	$ 144.00	$ 1,728.00	$ 82.00		$ 1,954.00	$ -	$ 1,504.00			$ 6,916.00	$ 3,908.00	3,008.00	$ 54.28
Host on-site evaluation	0	12	22	1	35				2	70				
	$ -	$ 864.00	$ 1,188.00	$ 41.00		$ 2,093.00	$ -	$ 15.00			$ 4,216.00	$ 4,186.00	30.00	$ 59.80
Perform and report 2 sets of ongoing performance tests (OPT)	0	4	23	3	30				2	60				
	$ -	$ 288.00	$ 1,242.00	$ 123.00		$ 1,653.00	$ -	$1,128.00			$ 5,562.00	$ 3,306.00	2,256.00	$ 55.10
									Total	234	$ 18,534.00	$13,210.00	$5,324.00	

O&M Costs to Perform and report initial performance tests (IPT): $188/sample X 8 IPT samples
O&M Costs to Perform and report 2 sets of ongoing performance tests (OPT): $188/sample X 3 OPT samples/set X 2 sets per year

Table 3. Agency Burden

Activity	Legal GS 15 $101.21/hr	Program Management GS 14 $80.93/hr	Clerical GS 2 $19.68/hr	Expert $119.00/hr	Management $102.00/hr	Technical $83.00/hr	Agency hrs/yr/resp	Labor cost/yr/resp	Capital Startup Cost	O & M Costs	Number of Labs	Total hrs/yr	Total Costs per Year	Labor Cost/Activity	O&M Cost/Activity
Review initial laboratory applications		2			1	7	10				2	20			
$		$ 161.86			$ 102.00	$ 581.00		$ 844.86		$5.00			$ 1,699.72	$1,689.72	$ 10.00
Review laboratory re-evaluation applications		2			1	14	17				21	357			
$		$ 161.86			$ 102.00	$ 1,162.00		$ 1,425.86		$5.00			$ 30,048.06	$29,943.06	$ 105.00
Conduct and review off-site re-evaluation activities		5			5	42	52				21	1092			
$		$ 404.65			$ 510.00	$ 3,486.00		$ 4,400.65		$20.00			$ 92,833.65	$92,413.65	$ 420.00
Conduct and review initial on-site evaluations		4.25		55	20	65	144				2	288.5			
$		$ 343.95		$ 6,545.00	$ 2,040.00	$ 5,395.00		$ 14,323.95		$3,350.00			$ 35,347.91	$28,647.91	$ 6,700.00
Conduct and review on-site re-evaluations		4.25		46	14	46	110				21	2315.3			
$		$ 343.95		$ 5,474.00	$ 1,428.00	$ 3,818.00		$ 11,063.95		$2,650.00			$ 287,993.00	$232,343.00	$ 55,650.00
Prepare and distribute spiking suspensions for IPTs; review IPT data		2	0.5		0.5	6	9				2	18			
$		$ 161.86	$ 9.84		$ 51.00	$ 498.00		$ 720.70		$375.00			$ 2,191.40	$1,441.40	$ 750.00
Prepare and distribute spiking suspensions for OPTs for initial labs; review OPT data		2	1		1	8	12				2	24			
$		$ 161.86	$ 19.68		$ 102.00	$ 664.00		$ 947.54		$375.00			$ 2,645.08	$1,895.08	$ 750.00
Prepare and distribute spiking suspensions for OPTs for each lab for each method version; review OPT data		3	3		1.5	11.5	19				67	1273			
$		$ 242.79	$ 59.04		$ 103.62	$ 954.50		$ 1,359.95		$ 200.00			$ 104,516.65	$91,116.65	$ 13,400.00
Coordinate follow-up activities for poor PT results		2			1	6	9				9	81			
$		$ 161.86	$ -	$ -	$ 102.00	$ 498.00		$ 761.86		$ -			$ 6,856.74	$6,856.74	$ -
											Total	5468.8	$ 564,132.21	$486,347.21	77,785.00

Total hrs/yr to Prepare and distribute spiking suspensions for OPTs for each lab for each method version; review OPT data: Costs and hours reflect 3 PT rounds per year for 63 labs and 4 labs analyzing an additional set of samples for each round.
O&M Costs to Conduct and review on-site re-evaluations: Travel costs

Table 4a. Total Respondent Burden

	Number of respondents	Number of Activities	Total hours/year	Total Labor cost/year	Total Annual Capital costs	Total Annual O&M Costs	Total Annualized Cost
Initial Evaluation	2	4	234	$ 13,210.00	$ -	$ 5,324.00	$ 18,534.00
Re-Evaluation	63	6	4609	$ 256,590.40	$ -	$ 136,605.00	$ 393,195.40
Total Burden	65	10	4843	$ 269,800.40	$ -	$ 141,929.00	$ 411,729.40

Table 4b. Total Agency Burden

	Number of respondents	Number of Activities	Total hours/year	Total Labor cost/year	Total Annual Capital costs	Total Annual O&M Costs	Total Annualized Cost
Total Burden	1	9	5469	$ 486,347.21	$ -	$ 77,785.00	$ 564,132.21