WI State Laboratory of Hygiene
***Cryptosporidium* Proficiency Testing Program**

General Program Information for Participant Laboratories

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1. Purpose

1.1. The *Cryptosporidium* Proficiency Testing Program (PT) has been designed to provide water testing laboratories and accreditation agencies with a means of assessing a laboratory's performance of U.S. EPA method 1622, 1623, or 1623.1 relative to other laboratories performing *Cryptosporidium* detection methods.

2. Scope

2.1. The organisms used in this PT program include: *Cryptosporidium parvum* (Iowa isolate) and *Giardia lamblia* (H3 isolate). Participants will provide numerical data after seeding three PT samples into bulk reagent water samples and processing the samples according to either method 1622, 1623, or 1623.1 for the recovery of *Cryptosporidium* (method 1622) or *Cryptosporidium* and *Giardia* (method 1623 or 1623.1). Laboratories new to the Program may be required to analyze eight samples and should consult their accreditation or certification agency to confirm the required number of samples. Although participants will not be scored on *Giardia* recoveries, *Giardia* is included both as an internal process control and to provide information to laboratories that test for *Giardia* and *Cryptosporidium* simultaneously. Participant laboratories are not required to process samples for recovery of *Giardia*.

3. Criteria for Participation

3.1. The PT Program is open to any laboratory interested in assessing their *Cryptosporidium* and *Giardia* testing performance. Only data sets that include results for all three samples and are submitted by a laboratory approved to perform method 1622, 1623, or 1623.1 by a recognized accreditation or certification body are included in the overall laboratory recovery statistics; however, data from non-approved laboratories will be analyzed on an individual basis and summary statistics describing their performance will be provided with their final report. Laboratories should note that methods 1622, 1623, and 1623.1 have very specific facility, personnel, and QA requirements. Refer to the method references in section 13 of this document.

3.2. Laboratories previously enrolled in the Program and any other laboratories expressing interest in the Program will be provided with the scheduled date 4-6 months in advance of an upcoming PT round. Laboratories must complete an enrollment form and pay the enrollment fee prior to participation. Laboratories may order more than one set for the same method however all results will be forwarded to their accreditation or certification agency. **Cancellations must be made 20 days prior to PT sample shipment.** No refunds will be issued to laboratories cancelling after this date.

4. Production and Quality Control of PT Materials

4.1. PT samples will be prepared twice annually using flow cytometry with cell sorting. Laboratories will receive PT samples containing live *Cryptosporidium parvum* oocysts

and *Giardia lamblia* cysts. Samples will be shipped in insulated containers with ice packs via overnight courier. Shipping will be performed by trained personnel and in accordance with international transport regulations. Additional shipping fees for shipments outside of the continental U.S. may be billed to the respective participant laboratory and overnight delivery of these shipments cannot be guaranteed. All customs fees, taxes, and duties for these shipments will be billed to the receiver.

4.2. Extensive quality assurance procedures are in place for each aspect of PT sample production. Organisms will undergo significant testing prior to use in a PT round. If any results from the pre-event screening or production procedures indicate that samples prepared with these organisms may not be fit for distribution, replacement samples will be prepared. Participants will be informed of the new shipment date, if different.

4.3. Protocols for homogeneity and stability testing are in place to ensure the quality of the samples and their integrity over the course of the PT event. Homogeneity will be assessed throughout the preparation event by performing on-going precision and accuracy checks. These checks will be performed by assessing the precision and accuracy of organism delivery prior to sample production and at intervals of every 10 samples. Dose uncertainty calculation procedures are in place. Organism stability will be assessed by analyzing randomly selected samples prepared and packed in a manner identical to that of routine samples. Samples will be analyzed by a subcontracted, accredited laboratory performing EPA Method 1623 or 1623.1.

5. Participant Information and Reporting

5.1. In addition to the PT samples, participant laboratories will be provided a list of contents, general instructions for seeding the organisms, a report form, and labels for return shipment of boxes and packing materials. Laboratories will process the samples according to the general instructions included with the PT samples using method 1622, 1623, or 1623.1. Laboratories must analyze samples in a manner consistent with routine samples. Laboratories must archive slides from both their PT samples and associated controls for 60 days.

5.2. When reporting results, laboratory personnel must sign a statement attesting that results were attained in their laboratory using standard procedures, and data was produced without collusion or consultation with other laboratories. Participant laboratories must return their results within 15 days after sample preparation. Late results will not be scored. The results package must include the results form, the attestation statement, and bench sheets for each sample and controls. Laboratories are cautioned not to commit transposition, typographical, recording, or other errors when transferring results from bench sheets to the PT Data Summary Form. If it is

determined that an error occurred, the affected sample(s) will be scored as zero(es). Submitted results are considered final. Results may not be retracted and resubmitted.

6. Data analysis and Reports

6.1. Analysis of the PT data will be performed using SigmaStat. Procedures for dealing with unacceptable data are in place. Data summaries including mean recovery and standard deviation will be calculated for: 1) each individual laboratory; 2) each concentration method; and 3) all approved laboratories.

6.2. Laboratory performance will be assessed by comparing the participant's mean recovery with the overall mean recovery and using previously published U.S. EPA acceptance criteria (Federal Register, Vol. 74, No. 36, Wednesday, February 25, 2009, pages 8529-8534). Laboratories with a mean recovery within 2 standard deviations (SD) of the mean recovery for all approved labs in a given round will have passed the event while those with recoveries below the 2 SD will fail the PT round. Laboratories whose recoveries were greater than 2 SD above the mean overall recovery or in excess of 100% ± the uncertainty measurement may be asked to send their slides (matrix blank, on-going precision and recovery, all PT samples slides) to the PT provider for examination and enumeration. Failure to submit PT slides within 1 week of the request will result in a failing score.

6.3. All laboratories will receive two reports: 1) the General PT Summary report which summarizes the overall recovery of approved laboratories; and 2) a participant laboratory-specific report which includes a cover letter stating their pass/fail status, a table showing round-specific performance, and a chart showing recovery means over time. Reports will be submitted to the participant laboratory and their accrediting or certifying agency simultaneously.

6.4. Reports will be issued within 6 weeks of the result submission deadline. Reports may be used to demonstrate acceptable performance to accreditation agencies. They are not an endorsement by the WSLH and may not be used as such.

7. Sample Replacement

7.1. Samples that arrive in an unusable condition or have been lost or misdirected by the courier will be replaced while those that have been mishandled by the participant laboratory may be replaced for a fee if replacement materials are available. Due to the short outdate of these samples, laboratories are encouraged to process samples **as soon as possible** so that replacement samples, if needed, can be shipped and processed prior to the sample outdate. The sample outdate is the Friday following a Monday shipment date. Samples cannot be shipped after Thursday in a PT production week.

8. Organization and Management

8.1. The WI State Laboratory of Hygiene (WSLH) is Wisconsin's Public Health Laboratory. The *Cryptosporidium* PT Program is operated by WSLH staff. The day to day operation of the PT Program is the responsibility of the PT Program coordinator with guidance from a proficiency testing advisory committee. Subcontracted activities include statistical analyses and pre-screen and stability testing analyses.

8.2. The PT Program is reviewed by the program coordinator, staff, and advisory committee following the completion of each PT event. Participants will have the opportunity to provide input through an online survey at the completion of each round. Participant laboratories are also encouraged to provide feedback at any time via email or telephone. Any changes in the Program will be conveyed to the participant laboratories through email or writing.

9. Confidentiality

9.1. Participant laboratories are assured strict confidentiality. Participant data will not be released without written authorization from the participant laboratory. Results may be released to the U.S. EPA or an accreditation agency upon written request by the participant laboratory.

10. Complaint/Appeals Procedure

10.1. Any complaint that cannot be resolved by the participant laboratory and the PT provider will be directed to a member of the PT Advisory Committee.

10.2. Participants have the right to appeal. All appeals must be submitted in writing by the laboratory director within two weeks of receiving results. Appeals that cannot be resolved by Program staff will be directed to an impartial third party for review.

11. Liability

11.1. In the unforeseen event of a PT Program-wide sample stability test failure detected after the PT samples have been processed, replacement samples will be provided at no cost. The WSLH *Cryptosporidium* PT Program will not provide compensation for any labor, reagent or other PT-related costs incurred by the participant laboratories.

12. Schedule for Key Activities

12.1. Requests for enrollment in the PT program are submitted (at least 20 days prior to each PT round).

12.2. Organism fitness for purpose is evaluated by the Analyte Screening Laboratory 14 days prior to the PT Event.

12.3. Samples are prepared and shipped to participant laboratories.

12.4. Participant laboratories analyze their samples according to their standard methods and within the expiration date listed on the PT sample tube.
12.5. Participants submit their results within 15 days after sample production.
12.6. Data are analyzed and reports are generated and validated.
12.7. Reports are issued within 6 weeks of result reporting deadline.
12.8. Electronic surveys are sent to participant laboratories.

13. Related Documents

13.1. Method 1622: https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf
13.2. Method 1623: https://www.epa.gov/sites/production/files/2015-07/documents/epa-1623.pdf
13.3. Method 1623.1: http://water.epa.gov/scitech/drinkingwater/labcert/upload/epa816r12001.pdf
13.4. Federal Register**,** Vol. 74, No. 36, Wednesday, February 25, 2009, pages 8529-8534.