***Cryptosporidium* Proficiency Test Program for Laboratories using US EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA, December 2005 version**

Preferable Specifications to Establish Proficiency Test (PT) Provider Accreditation

1. Distribute 2 sets of 3 PT samples for each participating laboratory every year.
2. Score PT results and track results over time.
3. Ensure that the oocysts supplied in the PT samples are robust enough to last the period of time required for the entire PT round.
4. Document accurate and precise calculation of the number of oocysts in samples.
5. Document that the specific isolate of oocysts used for each round can be recovered using Method 1623 at a percentage which is within the criteria for the Method.
6. Supply PT samples in a standardized matrix which simulates raw water and provides each laboratory with comparable method interference.

Example of Procedure to Ensure Quality of PT Samples

1. General overview:
    a. Distribute 3 PT samples for each laboratory, twice a year. Collect, validate, and manage laboratory sample results. Report statistical analysis of the results to the approval authority and laboratories. Maintain an advisory/steering group involved in program supervision and operation.
    b. Track laboratory contact information and test results
    c. Schedule PT rounds >2 months in advance of sample distribution in conjunction with an LT2-approved laboratory that screens and documents the oocyst integrity and recovery with Method 1623. This "Reference Laboratory" participates in PT rounds.
        i. *Cryptosporidium* oocysts from 2 different vendors should be tested separately in 10L reagent water (RW) and in 10L RW fortified with a standardized matrix. These experiments are single blind studies designed to mimic PT samples (or could be actual PT samples).
        ii. The "Reference Laboratory" should maintain a control chart of their ongoing precision and recovery per Method 1623 for 20 – 30 of the most recent samples (Figure 1). This chart should be updated for all Method 1623-related analyses, including PT-related analyses, performed in this laboratory. The data should be compared to the acceptance criteria established by the approval authority.
2. Document oocyst integrity
    a. Evaluate potential lots of *Cryptosporidium* oocysts prior to each PT round to ensure that the quality of the oocysts is sufficient to produce reliable PT results.
    b. Temperature recorders and instructions should be supplied for each oocyst vendor to initiate temperature tracking for the oocysts. The PT provider should download, maintain and analyze temperature data. The participating laboratories and approval authority should be notified of any temperature excursions outside 1 – 10 ºC for more than 2 hours during shed, preparation, and storage. Similar notification should be given for any temperature excursions outside of 1- 20 ºC during shipment.

c. Assess oocyst inclusion and exclusion of vital dye e.g. Propidium Iodide.
d. Examine oocysts with DIC microscopy for oocyst integrity and cleanliness of suspension.
    i. Apply 500 oocysts to a slide and dry without staining.
    ii. Characterize 20 oocysts for their condition with DIC and note empty, amorphous, internal structures, and overall oocyst quality.
e. Document acceptable FITC and DAPI fluorescence
    i. Apply 500 oocysts to a slide, dry and stain according to Method 1623.
    ii. Examine 20 oocysts and rate for FITC fluorescence on a scale of 1+ to 3+ fluorescence intensity (1+=light, 2+=medium, 3+=strong).
    iii. Rate DAPI fluorescence as positive (+) or negative (-). Positive DAPI characteristics are either brightly stained blue nuclei or bright blue diffuse staining. Negative DAPI characteristics are light blue or have no blue staining.
f. Process an aliquot of oocysts with Method 1623 in reagent water and reagent water fortified with a standard matrix before each PT round
    i. Evaluate 5 oocysts for FITC and DAPI as described above.
g. Documentation may be represented as a bar graph (Figure 2).

3. Document oocyst recovery from standardized matrices with Method 1623
    a. Perform analyses with selected oocysts prior to, or concurrent with, the 1st week of a given PT round.
        i. 1 Method Blank
        ii. 4 replicates of 10L of reagent water spiked with blind number between 50 and 200 oocysts from PT lot,
        iii. 5 ml of reagent water spiked with 50 to 200 oocysts and processed from IMS step through Method 1623,
        iv. 4 replicates of 10L of reagent water with standardized matrix added, spiked with a blind number between 50 and 200 oocysts from PT lot,
        v. 10 ml of reagent water with standardized matrix added and spiked with blind number of oocysts between 50 and 200 oocysts and processed from IMS step through Method 1623.
    b. Repeat analyses in 3.i after, or concurrent with, the last week of a given PT Round.
    c. The "Reference Laboratory" should maintain a control chart of their matrix spike recovery per Method 1623 for 20 – 30 of the most recent samples (Figure 3).
    d. The approval authority should be notified via email as soon as possible of any recoveries which are lower than the acceptance criteria or any abnormal observations. Corrective action should be initiated.
4. Flow cytometry
    a. Equipment should be maintained and calibrated per manufacturer instructions.
    b. Target numbers for oocyst counts should be randomly selected. PT count values should be kept confidential until all participating laboratories have generated their independent results.
    c. Spiking suspensions should be prepared using *Cryptosporidium* oocysts <2 months old, and *Giardia* cysts <2 weeks old.

d. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The oocyst and cyst levels used for the initial calibration are the same as the levels used for the spiking suspensions. Each initial calibration sample will be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the initial calibration should be performed again, until the RSD of the 10 counts is ≤ 2.5%.
e. When sorting the spiking suspensions for use in PT samples, ongoing calibration samples should be performed at a 10% frequency, at a minimum. The first run and every eleventh sample should be sorted directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the batch should discarded.
f. Method blanks should be prepared and examined at the same frequency as the ongoing calibration samples.

5. PT sample preparation and distribution
    a. Prepare blind samples in 50 mL conical tubes containing a standard reference material that is commercially available and target amounts of *Cryptosporidium* no more than 2 months old at the time of distribution.
    b. *Giardia* cysts should be included as internal control and no more than 2 weeks old at the time of distribution.
    c. Include traffic report, data summary, Method 1623 bench sheet, report forms, and instructions with sample shipment. Electronic communication should be used whenever possible.
    d. Maintain enough replicate spiking suspensions until completion of the PT Round to use in the event of accidental sample loss or for use as a quality check sample.
    e. Shipments should be prepared with ice packs and insulated containers according to IATA dangerous goods regulations by personnel trained to ship hazardous materials.
    f. The different participants should simultaneously receive test items, which are as similar as possible.
6. Data collection and communication with approval authority
    a. Data should be collected electronically from all participating laboratories, validated and analyzed using an automated data validation and reporting system.
    b. Laboratories that have submitted outlying data should be contacted within 10 working days in an attempt to identify whether the reason for unexpected results was testing error or laboratory error.
    c. The final report should be distributed via email within 25 business days of sample distribution. This report should include:
        i. Line graph plotting average PT recovery for all participating laboratories and the recovery from the individual laboratory (Figure 4).

ii. Results of the "Reference Laboratory's" quality control, oocyst assessments and PTs.
iii. Contact information for oocyst vendors, isolate/strain and lot numbers. Document confirmation of the strain and isolate (i.e. last sequence date).
iv. Host animal taxon, supplier, and brand name of feed, nutritional supplements and immunosuppressants.
v. Oocyst harvesting and purification procedure(s), including number of repetitions, dates of shedding, shipping, and receiving.
vi. Calibration values including mean and standard error for the oocyst counts and dates of calibration and enumeration.
vii. Storage suspension constituents (list antibodies and fungicides).
viii. Troubleshooting activities associated with the test process.
ix. Documentation of statistical procedures.

7. Performance assessment
    a. Participants are assessed on the basis of their results in one round of the same PTs.
    b. Performance is based on deviation from the assigned values (accuracy), and any pertinent technical considerations.
    c. Laboratories will be notified if their mean recovery was <2 standard deviations below the mean recovery for all approved laboratories participating in a given test event.
8. The provider sends the approval authority a list of laboratories that were notified that their mean recovery was outside 2 standard deviations below the mean recovery for all approved laboratories participating in a given test event.
9. The provider can supply participants with technical assistance to help improve their performance or provide contact information for technical instructions.
10. An approved laboratory may be downgraded to "Provisionally Approved" status for *Cryptosporidium* analysis for any of the following reasons:
    a. Failure to document a minimum of 22 percent for on-going precision and recovery values.
    b. Failure to submit valid PT results or meet PT acceptance limits described by the Approval Authority for the first 2 initial testing events or 2 out of 3 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.
    c. Failure to submit PT slides within 3 weeks of PT test event when requested by the Approval Authority.
    d. Failure to include all QA/QC results with PT results.
11. An approved laboratory, or provisionally approved, laboratory may no longer be approved for any of the following reasons:

a. Falsification of data or other deceptive practice.
b. Failure to submit valid PT results for the next 2 consecutive authorized PT events after downgrade to provisional status.
c. Failure to provide a letter to the Approval Authority within 30 days of downgrade that adequately explains what immediate corrective actions were taken.

12. Laboratories with grievances during the authorized PT events should immediately contact the Program Manager at the Approving Authority and try to remedy the problem. A documented procedure for the handling of complaints should be available.

Figure 1. Example of an OPR quality control chart

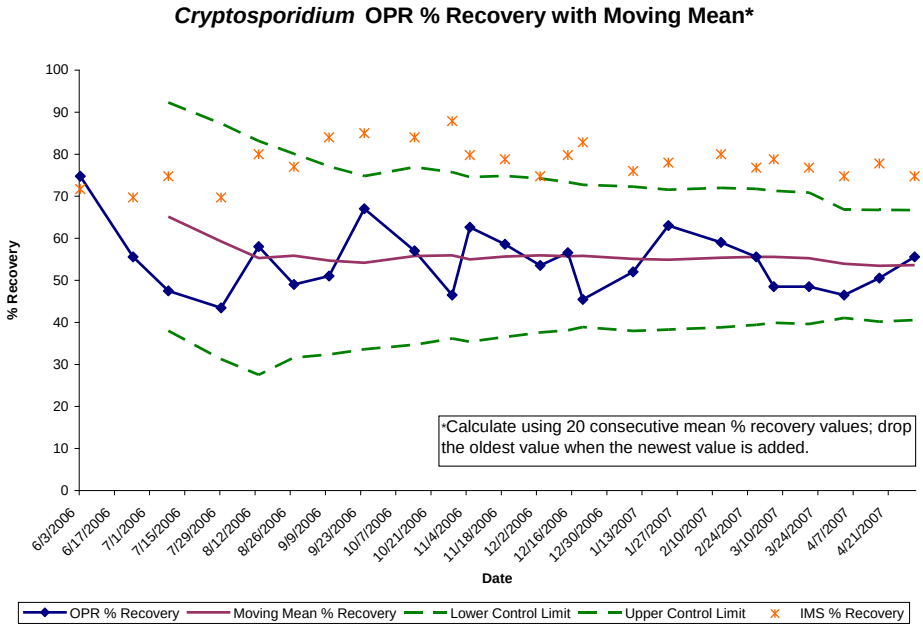

Figure 2. Example Comparing Characterizations for the PT Screen and the PT Round

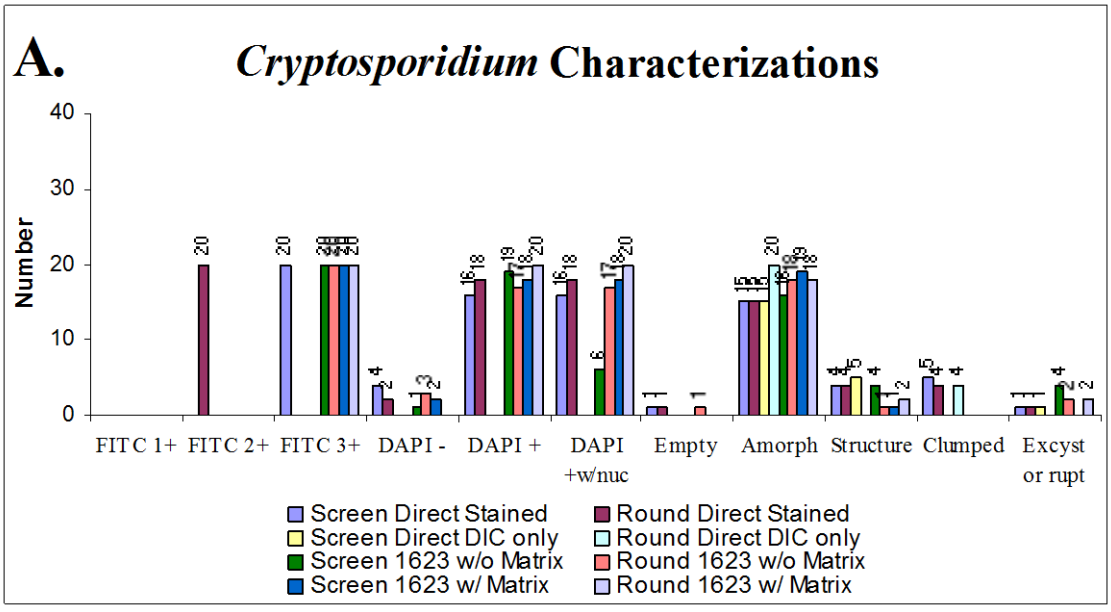

Figure 3. Example of MS quality control chart

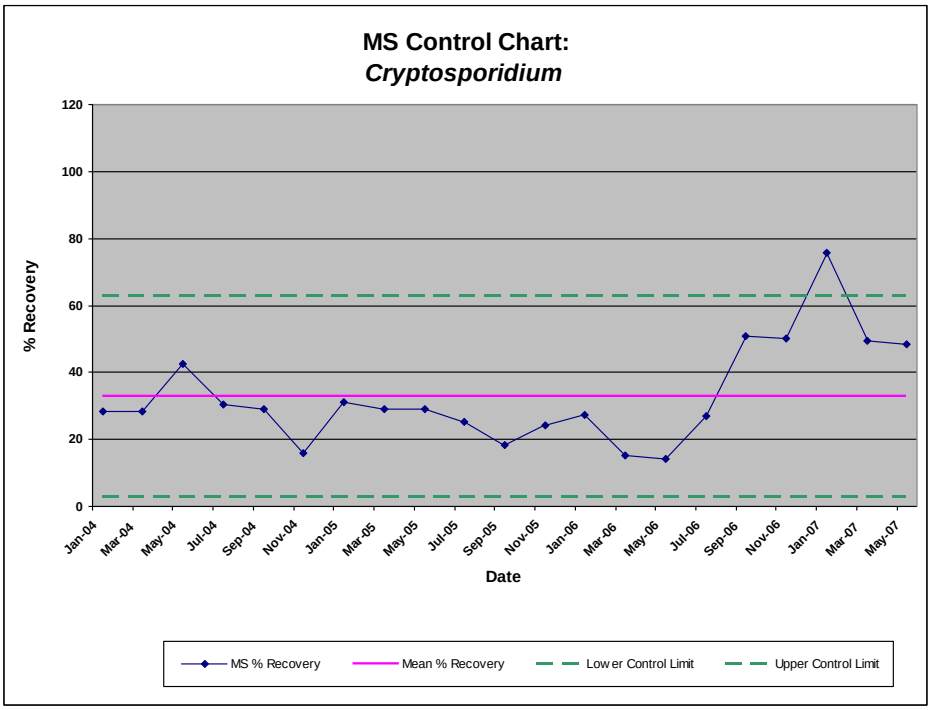

Figure 4. Example of PT graph