General Instructions

1. **Inspect samples immediately upon receipt.** The numbers on the sample tubes should match those listed under "Sample number" on your traffic report. Verify that the method indicated on the traffic report is the method you will use to process the samples. If you are missing samples or paperwork, or the information on the traffic report is incorrect, call (608) 224-6260.

2. **Sample replacement.** Leaking or missing samples? Call (608) 224-6260 for replacements. Mishandled samples may be replaced, if available, for a fee. **All requests for replacements must be made within 2 calendar days of the shipment date.**

3. **Sample Storage.** Immediately unpack samples from the shipping container.

3.1. **Refrigerate** samples (1-10^{o}C) until you are ready to begin filtration.

3.2. Process the samples by the "Use by" date listed on the sample tubes, using the method specified on the traffic report. **It is recommended that you process your samples as soon as possible so that a replacement set, if needed, may be shipped in a timeframe that allows you to analyze the samples prior to their outdate.**

3.3. **Proficiency testing samples must be tested in a manner identical to that of routine samples.** All holding times in Method 1622, 1623, or 1623.1 must be met. Run the positive and negative controls specified by the method.

4. **Recommended Procedure for Seeding PT Samples into Reagent Water Using Methods 1622/1623/1623.1.**

NOTE: PT samples contain live organisms. Proper biological safety precautions should be taken.

4.1. Arrange a 10L or 50L bottom-dispensing carboy to gravity-feed the inlet of the sampling filter. The self-priming centrifugal pump should be placed after the filter. If not using a carboy with a bottom spigot, then be sure that the end of the tubing is weighted to remain in the bottom of the carboy. A peristaltic pump may be placed before or after the sampling filter. Place a large sterile stir bar in the carboy.

4.2. Fill the carboy with 10L or 50L of reagent water. Place the 10L carboy on a stir plate and adjust so a vortex is created. For the 50L carboy, use a paddle or propeller and adjust the stirring to maintain good mixing without splashing.

4.3. Shake the PT sample well to mix the sample. Carefully pour the contents of the tube into the carboy. Add 20mL of reagent water to the tube, cap and vortex for 10 seconds. Add this rinsate to the carboy. Repeat with another 20mL of reagent water.

4.4. Allow the spiked sample to mix in the carboy for at least one minute. Remove an aliquot from the carboy and determine the turbidity. Return the aliquot to the carboy, rinsing the aliquot tube twice with reagent grade water.

4.5. Turn on the pump and allow the flow rate to stabilize. Set the flow at the rate specified by the method.

4.6. When the water reaches the bottom, tilt the carboy so that it is completely emptied. Turn off the pump.

4.7. Add one liter of reagent water to the 10L carboy (for larger carboys, add 2-3 liters). Swirl to rinse down the sides. Turn on the pump again to pull/push all the water through the filter. Turn off the pump.

4.8. Follow your routine method from this point on as though the PT were a field sample. (Either the December 2005 version of method 1622/1623 or the January 2012 version of method 1623.1 should be used.) Analyze the entire sample volume seeded and filtered.

4.9. The sampling apparatus and tubing should be thoroughly cleaned and disinfected between samples.

5. Reporting Results

5.1. Record the results of the PT samples and the associated OPR and MB samples on your own forms (bench sheet and examination form) along with enclosed PT Data Summary Form. **The data entered on the PT Data Summary Form is the data that will be used to score your laboratory. Be certain that you do not commit transposition errors when transferring the data from the bench sheet to the PT Data Summary form. Bench sheet and PT Data Summary Form comparisons may be performed. Please write legibly.**

5.2. **Complete the Attestation Form** (see the reverse side of the PT Data Summary form) stating that results are true and valid or qualify data as needed. **Note that the title "designee" refers to any person that <u>your</u> laboratory has authorized to release results.**

5.3. Submit results WSLH in one of the following manners: 1) electronically to **CryptoPT@slh.wisc.edu;** 2) or fax to **(608)226-5230; or 3) send hard copies to *Cryptosporidium* PT Results, Becky Hoffman, WSLH, 2601 Agriculture Drive, Madison, WI 53718.** Please use registered or priority mail which allows you to print/file proof of delivery. Be sure to retain a copy of your original forms. Results must be **received** at WSLH, no later than **<u>10/21/14.</u>** You do not need to mail hard copies if you've used electronic submission.

Verify that you have included the following:

5.3.1. Internal bench and examination forms

5.3.2. PT Data Summary Form

5.3.3. Attestation Statement

5.4. Return all the empty packaging (box, plastic can and Styrofoam container) and cold packs to **Marty Collins, 2601 Agriculture Dr., Room 100C, Madison, WI 53718** via UPS or regular mail as soon as possible.

5.5. Call 608-224-6260 or e-mail PT program staff at: CryptoPT@slh.wisc.edu with any questions regarding the PT samples or the PT Program.

5.6. ****NOTE: All slides for PT samples should be archived for at least 60 days. **<u>This includes associated OPR, MB, positive staining and negative staining control slides.</u>**

PT Data Summary Form

Results and Attestation form are due no later than 10/21/14.
CryptoPT@slh.wisc.edu

Laboratory ID: _____

Laboratory Name: _____

Date: _____

Filter Used: _____

Method version (indicate one): 1622 ☐ 1623 ☐ 1623.1 ☐

Note: Report as total cysts and oocysts in the PT sample (Check for transposition errors before submitting this report. This is data that will be used to score your laboratory.) Write legibly.

Sample Number	**Total *Cryptosporidium***		**Total *Giardia***		**Equivalent Sample Volume Analyzed**	**Turbidity (NTU) If measured**
Control Data	**Number Seeded**	**Number Counted**	**Number Seeded**	**Number Counted**	**Entire volume analyzed? Y/N**	
OPR						NA
Method Blank	0		0			NA

This document is date- specific and is updated for each PT event. Please ensure the date listed on the header corresponds with the most current round.

Attestation Statement:

I attest that the results reported on the PT Summary Form are true and valid. These results were obtained:

1. Using the method with which our laboratory analyzes routine samples;
2. Using the either the December 2005 version of US EPA method 1622/1623 or the January 2012 version of US EPA method 1623.1 (if results are to be submitted for approval purposes), and that all control performance and holding time criteria associated with the method were met.

Furthermore, results were not obtained through discussion or collaboration with persons outside the Laboratory.

***List the sample numbers to which this statement applies (or write "All"):** _____

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***Is your laboratory accredited/certified to perform the method used for this event?** _____

If yes, please list the accreditation/certification agency <u>and</u> the most recent audit date (month/year). _____

Director/Designee Signature: _____ Date_____

Print Name: _____

Analyst Signature: _____ Date_____

Print name: _____

Analyst Signature: _____ Date_____

Print name: _____

This document is date- specific and is updated for each PT event. Please ensure the date listed on the header corresponds with the most current round.