Checklist A – Method 1623/1623.1 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	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
1 Quality Assurance									
1.1 Is documentation (e.g., resume, sample list) available for all Method 1623/1623.1 staff?	9.1	9.1	1.	Requirement GLP					
1.1.1 Have technicians/analysts analyzed the required number of samples using Method1623 or1623.1?	22.2	22.2	1.1 -1.3	Requirement GLP					
1.2 Are employee training records available and up to date?	9.1	9.1	1.7	Critical GLP					
1.2.1 Have all analysts documented that they have read and understood the QA Plan and SOPs?	-	-	1.7	Critical GLP					
1.3 Is the laboratory performing analyst verification monthly and does the lab have corrective action procedures in place if criteria are not met?	10.6	9.10	7.1.9	Requirement					
1.3.1 If the laboratory has only one analyst, is the analyst demonstrating analyst verification through comparison with photo libraries or repetitive counts?	10.6.4	9.10.4	7.1.9.5	Recommendation					
1.4 Does the quality assurance plan address requirements for *Cryptosporidium* analysis under LT2ESWTR?				Critical					
1.5 Have acceptable initial precision and recovery analyses been performed for each version of the method the laboratory is using?	9.1.2.1.1	9.2	7.1.4	Requirement					

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1.6	Of the field/PT samples reviewed, is each field/PT sample associated with an acceptable method blank?		9.6.1	9.7	7.1.2 7.1.5.1	Requirement					
	1.6.1	Were all method blanks (MB) evaluated without contamination?	9.6.2.1	9.7.2	7.1.5	Requirement					# MB reviewed:
	1.6.2	Were the same lots of reagents (elution, IMS, and staining) used for the method blank and the associated field/PT samples?	-	-	7.1.5.3	Critical					
	1.6.3	Is method blank analyzed prior to the analysis of field/PT samples?	9.6	9.7	7.1.5.2	Requirement					
1.7	Is each field/PT sample associated with an acceptable ongoing precision and recovery (OPR) sample?		9.7	9.8	7.1.2 7.1.6.1	Requirement					
	1.7.1	What percentage of OPR samples evaluated met the recovery criteria?	9.7.3 Table 3 Table 4	9.8.3 Table 3 Table 4	7.1.6.2						# OPR reviewed:
	1.7.2	Were the same lots of reagents (elution, IMS, and staining) used for the OPR and the associated field/PT samples?	-	-	7.1.6.1	Critical					
	1.7.3	Is OPR analyzed prior to the analysis of field/PT samples?	9.7.1	9.8.1	7.1.6.2	Requirement					
	1.7.4	Does the laboratory maintain control charts of OPR results?	9.7.6 9.1	9.8.3 9.12.1 Table 2	7.1.7	1623 Recommendation 1623.1 Requirement					
	1.7.5	What is the mean and relative standard deviation (RSD), or standard deviation, of the recoveries of the OPR samples included in the control chart?	9.4.3 Table 3 Table 4	9.5.3 Table 3 Table 4	7.1.6.2	QC Criteria					Mean: RSD:
1.8	Were matrix spike (MS) samples analyzed at the minimum frequency of 1 MS per 20 (up to and including) field samples from each source?		9.1.8	9.6.1	7.1.2 7.1.10.2	Requirement					# MS reviewed:
	1.8.1	Were MS sample volumes within 10% of their associated field samples' volumes?	9.5.1	9.6.2	7.1.10.3	Requirement					
	1.8.2	Were MS samples analyzed at the same time and using the same method variation as their associated field samples?	Table 2	Table 2	7.1.10.1	Requirement					

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1.8.3 What is the mean and relative standard deviation of the MS samples reviewed?	Table 3 Table 4 Table 5	Table 3 Table 4 Table 6	-	QC Criteria					Mean: RSD:
1.8.4 Does the laboratory maintain control charts of MS results?	9.5.1.4 9.1	9.6.2.3 9.12.2 Table 2	7.1.10.4	1623 Recommendation 1623.1 Requirement					
1.9 Were OPR samples spiked with 100 - 500 organisms?	9.7	9.8	7.1.6.1	Requirement					
1.10 Does the laboratory perform IMS controls and maintain IMS control charts? If not, how do they troubleshoot low recoveries?	9.7.5.3	9.8.7.3 9.13	-	Recommendation					
1.11 Does the laboratory have an adequate record system for tracking samples, including unique ID, from collection through log-in, analysis, and data reporting?	-	-	8.0	Critical GLP					
1.12 Is the laboratory using the Method December 2005 version of Method 1623 or Method 1623.1 for LT2 samples?				Requirement					
2 Data Recording Procedures									
2.1 Is shipping information complete, i.e., time/date of sample collection, sampler's name, time/date of sample receipt, receiver's initials, sample condition?	8.1.3	8.1.3	8.5	Requirement					
2.1.1 Were all samples evaluated received at ≤20°C and not frozen?	8.1.3	8.1.3	6.3.3	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 1623/1623.1 bench sheets used to record sample processing data?	-	-	8.2	Recommendation					
2.4 Are all primary measurements during each step recorded, including all raw data used in calculations?	9.1.2.2.5	9.3.5	8.0	Requirement					
2.5 Technician/analyst, date, and time of elution is recorded?	12.2.6.2.1	12.2.7.1 12.3.2.1	8.7	Requirement					
2.6 Technician/analyst, date, and time of slide preparation is recorded?	13.3.3.11	13.3.3.11	8.7	Requirement					

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	1623	1623.1	Cert		Yes	No	NA	UNK	
2.7 Technician/analyst, date, and time of staining is recorded?	14.10	14.10	8.7	Requirement					
2.8 Are batch and lot numbers of reagents used in the analysis of the sample recorded?	-	-	8.7	Critical					
2.8.1 Lot number for the IMS kit is recorded?	-	-	8.7	Critical					
2.8.2 Lot number of the staining kit is recorded?	-	-	8.7	Critical					
2.8.3 Lot number of the spiking suspensions is recorded?	-	-	3.21.4 8.7	Critical					
2.9 Spike value recorded for all spiked samples?	-	-	3.21.4 8.6	Requirement					
2.10 Are Method 1623/1623.1 *Cryptosporidium* Slide Examination forms used to record sample examination results?	15.2	15.2	8.2	Requirement					
2.11 Name of examining analyst is recorded?	15.2.6	15.2.6	8.7	Requirement					
2.12 Date and time of sample examination is recorded?	15.2.4	15.2.4	8.7	Requirement					
2.13 Are calculations of final concentrations and recoveries complete and correct?	-	-	-	Requirement					
2.14 Is the size of the cysts and oocysts reported to the nearest 0.5 µm?	15.2.2.3 15.2.3.3	15.2.2.4 15.2.3.4	5.4.9.4 5.4.10.4	Requirement					
2.15 Is each reported positive organism detected in a field sample characterized and recorded?	15.2	15.2.2.1 15.2.3.1	5.4.9.1 5.4.10.1	Requirement					
2.16 Do values recorded on the data sheets match the values reported to the client?	-	-	8.1	Requirement					
2.17 Are mistakes on all forms crossed out with a single line, initialed, and dated?	-	-	8.2	Critical					
2.18 Are data always legible and recorded in pen?	-	-	8.2	Critical					
2.19 Was the final report reviewed by QA manager, lab director or an individual other than the analyst?	-	-	8.1	Critical					
2.20 Do records demonstrate each analyst's characterization of 3 oocysts and 3 cysts from positive control for each microscopy session?	15.2.1.1	15.2.1.1	5.4.6	Requirement					
2.21 Data shows that no more than 0.5 mL of pellet was used per IMS?	13.2.4	13.2.3	5.2.3 8.6	Requirement					

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3 Holding Times –Method 1623.1									
3.1 Is sample elution initiated within 96 hours of sample collection or field filtration?	8.2.1 Table 1	8.2.1 Table 5	6.4 8.7	Requirement					
3.2 Are sample elution, concentration, and purification steps completed in one work day?	8.2.2 Table 1	8.2.2 Table 5	6.4 8.7	Requirement					
3.3 Are slides stained within 72 hours of application of the purified sample to the slide?	8.2.3 Table 1	8.2.3 Table 5	6.4 8.7	Requirement					
3.4 Are stained slides read and confirmed within 7 days of staining? [Section 8.2.4 and Table 5]	8.2.4 Table 1	8.2.4 Table 5	6.4 8.7	Requirement					
4 Spike enumeration procedures									
4.1 Source of flow cytometry-enumerated spiking suspensions.	-	11.2	-						
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.	-	-	7.1.10.3						
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?	-	-	4.3.1	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?	-	-	4.3.1	Critical GLP					
5.1.2 Has the reagent water been tested annually for metals – Pb, Cd, Cr, Cu, Ni, Zn?	-	-	4.3.1	Critical GLP					

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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?	-	-	4.3.1	Critical GLP					
5.1.3 Is reagent water tested monthly for heterotrophic plate count?	-	-	4.3.1	Critical GLP					
5.1.3.1 Are the results for the heterotrophic plate count acceptable, < 500 CFU/mL?	-	-	4.3.1	Critical GLP					
5.1.4 Is still or DI unit maintained according to manufacturer's instructions?	-	-	4.3.3	Critical GLP					
5.2 pH meter									
5.2.1 Accuracy ± 0.1 units, scale graduations, 0.1 units?	-	-	3.1.1	Critical GLP					
5.2.2 Is a record maintained for pH measurements and calibrations?	-	-	3.1.4	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)?	-	-	3.1.4	Critical GLP					
5.2.4 Are all pH buffers dated when received and opened, and discarded before expiration date?	-	-	3.1.5	Critical GLP					
5.3 Balances (top loader or pan balance)									
5.3.1 Are balance calibrations verified monthly using 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.	-	-	3.2.2	Critical GLP					
5.3.2 Is correction data and Certificate of Traceability available for weights?	-	-	3.2.3	Critical GLP					
5.3.3 Is preventative maintenance conducted yearly at a minimum?	-	-	3.2.4	Recommendation GLP					
5.4 Temperature recording device									

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5.4.1	Are calibration of thermometers checked annually (dial thermometers quarterly) at the temperature used against a reference NIST thermometer or equivalent?	8.1.4	8.1.4	3.3.2	Requirement GLP					
5.4.2	Is the sample storage refrigerator able to maintain temperature of 1 to 10°C?	-	-	3.7.1	Critical GLP					
5.5 Micropipetters										
5.5.1	Have micropipetters been calibrated within the past year?	9.2.1	Appendix A	3.8.2	Requirement GLP					
5.6 Centrifuge										
5.6.1	Is a maintenance contract in place or internal maintenance protocol available?	9.1	9.1	3.15.5	Critical GLP					
5.6.2	Is the centrifuge calibrated yearly?	-	-	3.15.5	Critical GLP					
5.7 Autoclave										
5.7.1	Are date, contents, sterilization time and temperature, and technician initials recorded for each cycle?	-	-	3.5.3	Critical GLP					
5.7.2	Is a maximum registering thermometer or continuous monitoring device used during each autoclave cycle?	-	-	3.5.5	Critical GLP					
5.7.3	Is automatic timing mechanism checked with stopwatch quarterly?	-	-	3.5.6	Critical GLP					
5.7.4	Are spore strips or ampules used monthly to confirm sterilization?	-	-	3.5.5	Critical GLP					
6 Quality Assurance Manual										
6.1	Does the laboratory have a formal QA laboratory plan prepared and ready for examination?	9.1	9.1	4.5.1	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?			4.5.2	Recommendation					
6.2.1	Is the QA manager separate from the lab director?	-	-	4.5.2	Recommendation GLP					

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	1623	1623.1	Cert		Yes	No	NA	UNK	
6.3 Does the laboratory have a schedule and/or procedure for all preventative maintenance of equipment?	-	-	4.5.3	GLP					

Comments: