Checklist C – Method 1623/1623.1 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	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
1 Laboratory Facilities									
1.1 Does laboratory appear to have established appropriate safety and health practices prior to use of this method?	5.0	5.0	4.1	Critical					
1.2 Do all laboratory personnel wear gloves when handling biohazard and toxic compounds, and change gloves before touching other surfaces and equipment?	5.3 5.4	5.3	4.1.6	Critical GLP					
1.3 Does the laboratory disinfect bench surfaces before and after analyses?	-	-	4.1.3	Critical GLP					
1.4 Does the laboratory have adequate bench space to perform the method?	-	-	2.0	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?	7.3	7.3	4.3.1	Requirement					
2.2 Are all reagents clearly labeled with identity of reagent, date of preparation, technician initials, and expiration date?	-	-	4.2.2	Critical GLP					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
2.3 Are SOPs available in the work area, and does laboratory practice reflect written procedures?				Critical GLP					
3 Sample Spiking				**Technician:**					
3.1 Was spike suspension vial vortexed for 30 seconds or per manufacturer's instructions?	11.4.3.1.2	11.2.3.2	-	Method Procedure					
3.2 Is the carboy used for method blank randomly selected from carboy stock to check efficacy of cleaning system?	-	-	7.1.5.3	Critical GLP					
3.3 Was the suspension vial adequately rinsed?	11.4.3.1	11.2.3	-	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 ® HV filtration				**Technician:**					
4.1.1 Are all components required for sample filtration present and in good condition?	6.1 6.2.1-6.2.2 6.3	6.1 - 6.2.8	6.1.7	Requirement GLP					
4.1.2 Is the filter assembly set up correctly?	Figure 3a	Figure 1	-	Method Procedure GLP					
4.1.3 Is the pump adequate for needs?	6.3.3	6.2.4	-	Requirement GLP					
4.1.4 Is the appropriate flow rate maintained (approximately 2 L/min)?	12.2.1.2	12.2.1.2	-	Method Procedure					
4.1.5 Is the volume filtered measured using a flow totalizer or calibrated carboy?	12.2.4.2	12.2.4.2	-	Requirement					

Item to be evaluated		Reference*			Classification	Satisfactory				Comments/ Response Requested
		1623	1623.1	Cert		Yes	No	NA	UNK	
4.1.6	Is the system well maintained and cleaned appropriately following use?	4.5	4.5	6.1.7	Critical GLP					
4.1.7	Is the system able to maintain seal during use with no leaks?	-	-	6.1.7	Requirement GLP					
4.1.8	Are SOPs for Envirochek® HV filtration available in the work area, and does laboratory practice reflect written procedures?				Critical GLP					
4.1.9	Other than issues noted for items 4.1.1 through 4.1.8 (if any) was Envirochek® HV filtration demonstrated successfully?									
4.2 Envirochek® HV capsule filter elution					**Technician:**					
4.2.1	Is the elution buffer prepared as per Method?	7.4.1	7.6.1	-	Method Procedure					
4.2.2	Is the wrist-shaker assembly set up correctly with arms fully extended?	12.2.6.1.1	12.2.6.1	3.14.2	Method Procedure GLP					
4.2.3	Is the dispersant addition performed as per Method 1623.1?	-	12.2.7	-	1623 Recommendation **1623.1 Method Procedure**					
4.2.4	Is volume of elution buffer measured to ensure the use of one 250 mL centrifuge tube?	12.2.6.2.2	12.2.8.2	-	Method Procedure					
4.2.5	Are the samples shaken at an appropriate speed?	12.2.6.2.3	12.2.8.3	3.14.3	Method Procedure					
4.2.6	Are the samples shaken three times for 5 minutes each time, and each in a different orientation?	12.2.6.2	12.2.8	-	Method Procedure					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
4.2.7 Are SOPs for Envirochek® HV 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® HV 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?	6.1 6.2.1 6.2.3 6.3	6.1 6.2.1-6.2.7 6.2.9	6.1.7	Requirement GLP					
4.3.3 Is the filter assembly set up correctly?	Figure 3b	Figure 2	-	Method Procedure GLP					
4.3.4 Is appropriate flow rate maintained of <4 L per minute for Filta-Max®?	12.3.1.1.3	12.3.1.1.3	-	Method Procedure					
4.3.5 Is the volume filtered measured correctly using a flow meter or calibrated carboy?	12.3.1.5.2	12.3.1.5.2	-	Requirement GLP					
4.3.6 Is system well maintained and cleaned appropriately following use?	12.3.4	12.3.4	6.1.7	Requirement GLP					
4.3.7 Is system able to maintain seal during use with no leaks?	-	-	6.1.7	Requirement GLP					
4.3.8 Does the laboratory indicate on the filter housing the correct direction of flow?	12.3.1.3	12.3.1.3	-	Critical					

Item to be evaluated		Reference*			Classification	Satisfactory				Comments/ Response Requested
		1623	1623.1	Cert		Yes	No	NA	UNK	
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?	12.3.2.1	12.3.2.1	-	Requirement GLP					
4.4.3	Is residual suspension rinsed from all containers?	12.3.2.2.1d	12.3.2.2.1d	-	Critical					
4.4.4	Is PBST used to elute the filter?	7.4.2.4	7.6.2.4	-	Method Procedure					
4.4.5	Is an appropriate amount of PBST used for each wash? (approx. 600 mL)	12.3.2.2	12.3.2.2	-	Method Procedure					
4.4.6	During the first wash, is the plunger moved up and down 20 times?	12.3.2.2.1h	12.3.2.2.1h	-	Method Procedure					
4.4.7	Is the plunger moved up and down gently to avoid generating excess foam?	12.3.2.2.1h	12.3.2.2.1h	-	Method Procedure					
4.4.8	During the second wash, is the plunger moved up and down 10 times?	12.3.2.2.2b	12.3.2.2.2b	-	Method Procedure					
4.4.9	If the automatic washer is used, is the machine operating properly?	12.3.2.1	12.3.2.1	-	Requirement					
4.4.10	Is the wash station cleaned adequately between samples?	12.3.4.2	12.3.4.2	-	Requirement GLP					

Item to be evaluated		Reference*			Classification	Satisfactory				Comments/ Response Requested
		1623	1623.1	Cert		Yes	No	NA	UNK	
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?									
5 Concentration										
5.1 Filta-Max® filter sample concentration					**Technician:**					
5.1.1	Is concentrator set up correctly?	12.3.3.2.1b	12.3.3.2.1a	-	Requirement GLP					
5.1.2	Is the force of the vacuum maintained below 30 cm Hg?	NOTE pg 43	NOTE pg 34	-	Method Procedure					
5.1.3	Is concentration performed after each of the washes?	12.3.2.2.1j	12.3.2.2.1j	-	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)?	12.3.3.2.1c	12.3.3.2.1b	-	Method Procedure					
5.1.5	Are the stir bar and concentration tube rinsed after each concentration and the liquid added to the concentrate?	12.3.3.2.1c	12.3.3.2.1b	-	Requirement					
5.1.6	Was the filter membrane washed twice with 5 mL of PBST?	12.3.3.2.3	12.3.3.2.3	-	Method Procedure					
5.1.7	Are SOPs for Filta-Max® filter sample concentration available in the work area, and does laboratory practice reflect written procedures?				Critical GLP					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
5.1.8 Other than issues noted for items 5.1.1 through 5.1.7 (if any) was Filta-Max® filter sample concentration demonstrated successfully?									
5.2 Envirochek® HV 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?	13.2.1 and NOTE pg 46	13.2.1 and NOTE pg 37	-	Method Procedure GLP					
5.2.2 Are the centrifuge tubes properly balanced prior to centrifugation?	-	13.2.1	3.15.4	Critical					
5.2.3 Does lab have easily accessible method for determining relative centrifugal force of centrifuges?	-	-	3.15.1	Critical GLP					
5.2.4 Is the sample centrifuged for 15 minutes, with time beginning when centrifuge reaches desired speed?	13.2.1	13.2.1	-	Method Procedure					
5.2.5 Is the centrifuge slowly decelerated at the end without the brake?	13.2.1	13.2.1	-	Method Procedure					
5.2.6 Is the pellet volume determined?	13.2.1	13.2.1	5.2.3	Requirement					
5.2.7 Is there a set of standards for comparison of pellet size?	-	-	5.2.3	Recommendation GLP					
5.2.8 Are SOPs for Envirochek® and Filta-Max® filter sample centrifugation available in the work area, and does laboratory practice reflect written procedures?				Critical GLP					
5.2.9 Other than issues noted for items 5.2.1 through 5.2.8 (if any) was Envirochek® HV or Filta-Max® filter sample centrifugation demonstrated successfully?									

Item to be evaluated		Reference*			Classification	Satisfactory				Comments/ Response Requested
		1623	1623.1	Cert		Yes	No	NA	UNK	
6	**Purification and Slide Preparation**				**Technician:**					
6.1	Is an approved IMS kit/manufacturer used?	7.5	7.7.1	-	Method Procedure GLP					
6.2	Is the supernatant from the centrifuged sample aspirated no lower than 5 mL of supernatant above every 0.5 mL pellet or portion of 0.5 mL pellet?	13.2.2	13.2.2 13.2.3	5.2.2 5.2.3	Requirement					
6.2.1	Are the samples aspirated using the pipette, with the documented internal diameter, as specified in the SOP?	-	NOTE pg 37	-	Critical					
6.2.2	Is the proper rate (mL/min) or pressure (psi) maintained throughout aspiration?	-	13.2.2 13.2.3	-	Method Procedure					
6.3	Is the pellet vortexed a sufficient time for resuspension?	13.2.3 13.2.4.1.3 13.2.4.2	13.2.2.1 13.2.3.1.2 13.2.3.2	-	Method Procedure					
6.4	Is the resuspended pellet volume quantitatively transferred to the flat-sided tube (2 rinses)?	13.3.2.1	13.3.2.1	-	Method Procedure					
6.5	Are the IMS beads thoroughly resuspended prior to addition to the flat-sided tube?	13.3.2.2 13.3.2.4	13.3.2.2 13.3.2.4	-	Method Procedure					
6.6	Is the flat-sided tube rotated at 18 rpm for 1 hour at room temperature?	13.3.2.6	13.3.2.6	-	Method Procedure					
6.7	Is the rotating mixer calibrated annually?	-	-	3.17.4	Critical GLP					
6.8	Is flat-sided tube correctly placed in magnet and rocked through 90 degrees about once per second?	13.3.2.7-13.3.2.9	13.3.2.7-13.3.2.9	-	Method Procedure					
6.9	Is all the liquid removed when decanting is performed with the magnet up?	13.3.2.11	13.3.2.11	-	Method Procedure					
6.10	Is the sample quantitatively transferred from the flat-sided tube to the microcentrifuge tube (2 rinses)?	13.3.2.13	13.3.2.14	-	Method Procedure					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	**1623.1**	**Cert**		**Yes**	**No**	**NA**	**UNK**	
6.11 Are the beads rinsed with PBS while inside the microcentrifuge tube?	13.3.4	13.3.2.17	-	1623 Recommendation 1623.1 Requirement					
6.12 Is standard NaOH (5 µL, 1N) and standard HCl (50 µL, 0.1N) used?	NOTE pg 49 & 50	NOTE pg 42	3.17.5	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?	13.3.3.2-13.3.3.4	13.3.3.2-13.3.3.4	-	Method Procedure					
6.14 Is a second dissociation performed?	13.3.3.10 NOTE pg 49	13.3.3.10 NOTE pg 41	5.2.4	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?	13.3.3.10	13.3.3.10 13.4.5	-	Circle one: A B					
6.16 Are the slides clearly labeled so they can be associated with the correct sample?	13.3.3.7	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?	13.3.3.12	13.3.3.12	-	Circle one: A B C					
6.19 If the slide is warmed, is incubator or slide warmer calibrated and labeled?	-	-	3.4	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?	14.2	14.2	3.18.1	GLP					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
7.2 Is FITC stain applied according to manufacturer's directions?	14.2	14.2	5.3.2	Method Procedure					
7.3 Are positive and negative staining controls performed?	14.1	14.1	5.3.5	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?	14.3	14.3	5.3.3	Method Procedure					
7.5 Are the labeling reagents rinsed away properly after incubation, without disturbing the sample?	14.5	14.5	-	Method Procedure					
7.6 Was the working DAPI stain prepared the day it was used?	7.7.2	7.9.2	3.19.2	Method Procedure					
7.7 Is stock DAPI stored at 1 to 10°C in the dark?	7.7.1	7.9.1	3.19.1	Method Procedure					
7.8 Is the DAPI stain applied properly and allowed to stand for a minimum of 1 minute?	14.6	14.6	-	Method Procedure					
7.9 Is the DAPI stain rinsed away properly without disturbing the sample?	14.7	14.7	-	Method Procedure					
7.10 Is the mounting media applied properly?	14.8	14.8	-	Method Procedure					
7.10.1 What type of mounting media is used?	7.8	7.10	-	GLP					
7.10.2 Are all the edges of the cover slip sealed well with clear fingernail polish, unless Elvanol® is used?	7.9 14.9	7.11 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 Elvanol®)?	14.10	14.10	5.3.6	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?									

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
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?	6.9.2	6.7.2	3.22.3	Requirement GLP					
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?	6.9.3	6.7.3	3.22.3	Requirement GLP					
8.3 Does the microscope have appropriate objectives and filters for DIC, which change easily to and from epifluorescence?	6.9.1	6.7.1	3.22.4	Requirement GLP					
8.4 Are all portions of the microscope, from the light sources to the oculars, properly adjusted?	10.3	10.0 Appendix B	3.22.6	Requirement					
8.5 Is the DIC image appropriate for each laboratory microscope?	-	Figure 4	Visual Guide	Requirement					
8.6 Is microscope cleaned after every session?	10.4	10.9.8	3.22.11	Requirement GLP					
8.7 Does the microscope have a 20X scanning objective?	6.9.1	6.7.1	3.22.8	Requirement GLP					
8.8 Does the microscope have a 100X oil immersion objective?	6.9.1	6.7.1	3.22.8	Requirement GLP					
8.9 Is the microscope equipped with an ocular micrometer?	6.9.1	6.7.1	3.22.9	Requirement GLP					
8.10 Is a stage micrometer available to laboratory?	6.9.1 10.3.5	6.7.1 App. B 3	3.22.9	Requirement					
8.11 Is a calibration table for 100X objective located close to the microscope(s)?	10.3.5.7	App. B 3.7	3.22.9	Requirement					
8.12 Has the mercury bulb been used less than the maximum hours recommended by the manufacturer?	10.3.2.11	App.B 1.11	3.22.12	Requirement					

Item to be evaluated	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
8.13 Does the laboratory have a preventative maintenance agreement in place to service the microscope annually?	-	-	3.22.6	Critical GLP					
8.14 Are SOPs for sample examination available in the work area, and does laboratory practice reflect written procedures?				Critical GLP					
8.15 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 laboratory's positive staining control slide contain (oo)cysts at the appropriate fluorescence intensity for FITC?	15.2.1.3	15.2.1.3	5.4.8 5.4.9.2 5.4.10.2	Requirement					
9.2	Does the laboratory's positive staining control slide contain (oo)cysts at the appropriate fluorescence intensity for DAPI?	15.2.1.3	15.2.1.3	5.4.8 5.4.9.3 5.4.10.3	Requirement					
9.3	Does the laboratory's positive staining control slide contain an appropriate level of background fluorescence?	-	-	5.4.3	Recommendation					
9.4	Is concentration of oocysts on the positive staining control slide appropriate?	14.1.1 15.2.1.3	14.1.1 15.2.1.3	7.1.8.1	Requirement					
9.5	Does the laboratory's positive staining control exhibit appropriate contrast and organism features by DIC?	-	Figure 4	Visual Guide	Requirement					
9.6	Does the laboratory's OPR slide contain (oo)cysts at the appropriate fluorescence intensity for FITC?	15.2.2.1 15.2.3.1	15.2.2.2 15.2.3.2	5.4.9.2 5.4.10.2	Requirement					
9.7	Does the laboratory's OPR slide contain (oo)cysts at the appropriate fluorescence intensity for DAPI?	15.2.2.2 15.2.3.2	15.2.2.3 15.2.3.3	5.4.9.3 5.4.10.3	Recommendation					
9.8	Does the laboratory's OPR slide contain an appropriate level of background fluorescence?	-	-	5.4.3	Requirement					
9.9	Does the laboratory's OPR slide exhibit appropriate contrast and organism features by DIC?	9.7.1.1	9.8.1.1 Figure 4	Visual Guide	Requirement					
9.10	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?	10.6.3.1	9.10.3.1	7.1.9.4	Requirement					

Comments:

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 Onsite Sample Processing and Blind Spike Results – Comments and Recommendations		
Classification	**Comments**	**Response Requested**

13 Was analyst microscope operation acceptable? (yes/no)				
		Requirement *Method 1623: 10.3.4.1* *Method 1623.1: 10.7.1*	**Requirement** *Method 1623: 10.3.4.2-3* *Method 1623.1: 10.7.2-3*	**Requirement** *Method 1623: 10.3.6* *Method 1623.1: 10.8*
Name	**Position**	**Adjust Interpupillary Distance**	**Focus both eyepieces**	**Establish Kohler Illumination**

14 Slide Count and Analyst Verification Results (yes/no)					
	Requirement *Method 1623: 10.6.3.1* *Method 1623.1: 9.10.3.1*	**Requirement** *Method 1623: 10.6.3.1* *Method 1623.1: 9.10.3.1*	**Requirement** *Method 1623: 15.2* *Method 1623.1: 15.2*	**Requirement** *Method 1623: 15.2.2.3 15.2.3.3* *Method 1623.1: 15.2.2.4 15.2.3.4*	**Requirement** *Method 1623: 15.2.2.3 15.2.3.3* *Method 1623.1: 15.2.2.4 15.2.3.4*
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		
Classification	**Comments**	**Response Requested**