Checklist B - Laboratory SOP 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 For each item, does the SOP specify:	Reference*			Classification	Satisfactory				Comments/ Response Requested
	1623	1623.1	Cert		Yes	No	NA	UNK	
1 Sample Spiking									
1.1 The suspension vial is vortexed for 30 seconds or per manufacturer's instructions?	11.4.3.1.2	11.2.3.2	-	Method Procedure					
1.2 The carboy used for the method blank is randomly selected from carboy stock to check efficacy of cleaning system or disposable carboys are used for all samples?	-	-	7.1.5.3	Critical					
1.3 The details of the suspension vial rinse, including volumes?	11.4.3.1	11.2.3	-	Method Procedure					
1.4 Acceptable sample spiking procedures, including issues not noted in items 1.1 through 1.3?				Critical GLP					
2 Filtration/Elution									
2.1 Envirochek® HV filtration									
2.1.1 The flow rate is maintained at approximately 2 L/min?	12.2.1.2	12.2.1.2	-	Method Procedure					
2.1.2 The volume filtered is measured using a flow totalizer or calibrated carboy?	12.2.4.2	12.2.4.2	-	Requirement					
2.1.3 The sample is stirred during filtration?	12.2.4.1	12.2.4.1	-	Method Procedure					
2.1.4 The details of the carboy rinse after filtration including volume?	12.2.4.5	12.2.4.6	-	Method Procedure					

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2.1.5	Appropriate maintenance and cleaning procedures?	-	-	-	Critical					
2.1.6	Acceptable Envirochek® filtration procedures, including issues not noted in items 2.1.1 through 2.1.5?				Critical GLP					
2.2 Envirochek® HV capsule filter elution										
2.2.1	Measurement of the volume of the elution buffer used or that the volume covers the membrane?	12.2.6.2.2	12.2.8.2	-	Method Procedure					
2.2.2	The speed that samples are shaken?	12.2.6.2.3	12.2.8.3	-	Method Procedure					
2.2.3	The dispersant is added to the sample as per Method 1623.1?		12.2.7	-	1623 Recommendation 1623.1 Requirement					
2.2.4	The samples are shaken three times for 5 minutes each time, and each in a different orientation?	12.2.6.2	12.2.8	-	Method Procedure					
2.2.5	Procedures for filter capsule rinse and addition of rinsate to the centrifuge bottle?	12.2.6.2.8	12.2.8.8	-	Method Procedure					
2.2.6	Acceptable Envirochek® capsule filter elution procedures, including issues not noted in items 2.2.1 through 2.2.5?				Critical GLP					
2.3 Filta-Max® filtration										
2.3.1	The flow rate is maintained at ≤4 L per minute for Filta-Max®?	12.3.1.1.3	12.3.1.1.3	-	Method Procedure					
2.3.2	The volume filtered is measured using a flow totalizer or calibrated carboy?	12.3.1.5.2	12.3.1.5.2	-	Requirement					
2.3.3	Appropriate maintenance and cleaning procedures? [Section 12.3.4]	12.3.4	12.3.4	-	Requirement					
2.3.4	Acceptable Filta-Max® filtration procedures, including issues not noted in items 2.3.1 through 2.3.3?				Critical GLP					
2.4 Filta-Max® filter wash station elution										
2.4.1	The use of PBST to elute the filter?	7.4.2.4	7.6.2.4	-	Method Procedure					

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2.4.2	The amount of PBST used for each wash? (approx. 600 mL)	12.3.2.2	12.3.2.2	-	Method Procedure					
2.4.3	The plunger is moved up and down 20 times during the first wash?	12.3.2.2.1 h	12.3.2.2.1 h	-	Method Procedure					
2.4.4	The plunger is moved up and down gently to avoid generating excess foam?	12.3.2.2.1 h	12.3.2.2.1 h	-	Method Procedure					
2.4.5	That during the second wash the plunger is moved up and down 10 times?	12.3.2.2.2 b	12.3.2.2.2 b	-	Method Procedure					
2.4.6	The instructions for cleaning the wash station between samples?	12.3.4.2	12.3.4.2	-	Requirement					
2.4.7	The housing is rinsed after filter is removed and the rinse is included in the sample volume?	12.3.2.2.1 d	12.3.2.2.1 d	-	Method Procedure					
2.4.8	Acceptable Filta-Max® filter wash station elution procedures, including issues not noted in items 2.4.1 through 2.4.7?				Critical GLP					
3 Concentration										
3.1 Filta-Max® filter sample concentration (as an alternative or in addition to Section 3.2)										
3.1.1	The force of the vacuum is maintained below 30 cm Hg?	NOTE pg 43	NOTE pg 34	-	Method Procedure					
3.1.2	That concentration is performed after each of the washes?	12.3.2.2.1 j	12.3.2.2.1 j	-	Method Procedure					
3.1.3	The sample is concentrated so that some liquid remains above the filter (enough to cover the stir bar about half-way)?	12.3.3.2.1 c	12.3.3.2.1 b	-	Method Procedure					
3.1.4	The stir bar and concentration tube are rinsed after each concentration and the liquid added to the concentrate?	12.3.3.2	12.3.3.2	-	Requirement					
3.1.5	The filter membrane is washed twice with 5 mL of PBST each time?	12.3.3.2.3	12.3.3.2.3	-	Method Procedure					
3.1.6	Acceptable Filta-Max® filter sample concentration procedures, including issues not noted in items 3.1.1 through 3.1.5?				Critical GLP					
3.2 Envirochek® HV and Filta-Max® filter sample centrifugation										

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		1623	1623.1	Cert		Yes	No	NA	UNK	
3.2.1	The sample is centrifuged at 1500 x G (maximum 2000 x G) using a swinging bucket rotor?	13.2.1 including NOTE	13.2.1 including NOTE	-	Method Procedure					
3.2.2	Instructions to ensure the centrifuge tubes are properly balanced prior to centrifugation?	-	-	3.15.4	Critical					
3.2.3	The sample is centrifuged for 15 minutes with start time beginning when centrifuge reaches the required speed?	13.2.1	13.2.1	-	Method Procedure					
3.2.4	The centrifuge is slowly decelerated at the end without using the brake?	13.2.1	13.2.1	-	Method Procedure					
3.2.5	Acceptable Envirochek® HV and Filta-Max® filter sample centrifugation procedures, including issues not noted in items 3.2.1 through 3.2.4?				Critical GLP					
4	**Purification and Slide Preparation**									
4.1	The centrifuged sample supernatant is aspirated no lower than 5 mL of supernatant above every 0.5 mL of the pellet or portion of 0.5 mL pellet?	13.2.2	13.2.2 13.2.3	5.2.2 5.2.3	Requirement					
4.1.1	The type and internal diameter of pipette used for aspiration of supernatant?	-	NOTE pg 37	-	Recommendation					
4.1.2	The rate of aspiration (i.e., mL/ min or pressure of the vacuum)?	-	13.2.2	-	Recommendation					
4.2	The tube is vortexed vigorously until pellet is completely resuspended?	13.2.3	13.2.2.1	-	Method Procedure					
4.3	Appropriate procedures for dividing pellets greater than 0.5 mL into subsamples and the analysis of the subsamples?	13.2.4	13.2.3	-	Critical					
4.4	No more than 0.5 mL of pellet is used per IMS?	13.2.4	13.2.3	5.2.3	Method Procedure					
4.5	The resuspended pellet volume is quantitatively transferred to the flat-sided tube (2 rinses) including the determination of the rinse volumes?	13.3.2.1	13.3.2.1	-	Method Procedure					
4.6	SL-Buffer A is used at room temperature or that it is checked for precipitate before use?	NOTE pg 47	NOTE pg 39	3.17.2	Method Procedure					
4.7	The volume of 10x SL-Buffer A is 1 mL?	13.3.1.2	13.3.1.2	5.2.5	Method Procedure					

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	1623	1623.1	Cert		Yes	No	NA	UNK	
4.8 The volume of 10x SL-Buffer B is 1 mL?	13.3.1.3	13.3.1.3	5.2.5	Method Procedure					
4.9 Instructions for thorough resuspension of IMS beads 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					
4.10 100 µL of *Cryptosporidium* and *Giardia* beads are used?	13.3.2.3 13.3.2.5	13.3.2.3 13.3.2.5	5.2.5	Method Procedure					
4.11 The flat-sided tube is rotated at 18 rpm for 1 hour at room temperature?	13.3.2.6	13.3.2.6	-	Method Procedure					
4.12 Which magnetic concentrators, MPC®-1 or MPC®-6, are used?				Method Procedure					
4.13 The placement of the flat-sided tube in the magnet and the rock technique and time?	13.3.2.9	13.3.2.8 13.3.2.9	-	Method Procedure					
4.14 The sample is quantitatively transferred from the flat-sided tube to the microcentrifuge tube (2 rinses) including rinse volumes?	13.3.2.13	13.3.2.14	-	Method Procedure					
4.15 The flat-sided tube is allowed to sit one minute after each transfer to accumulate residual sample, then the residual is transferred to microcentrifuge tube?	13.3.2.13	13.3.2.14	-	Method Procedure					
4.16 The magnet is in the vertical position in the MPC®-S?	-	13.3.2.13	-	Method Procedure					
4.17 The beads are rinsed with PBS while inside the microcentrifuge tube?	13.3.4	13.3.2.17	-	1623 Recommendation 1623.1 Requirement					
4.18 Standard NaOH (5 µL, 1N) and standard HCl (50 µL, 0.1N) are used?	NOTES pg 49-50	NOTES pg 42	3.17.5	Requirement					
4.19 The sample is 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	13.3.3	-	Method Procedure					
4.20 The magnet is in the slanted position in the MPC®-S for dissociation steps?	-	13.3.3.6	-	Method Procedure					
4.21 A second dissociation is performed?	13.3.3.10	13.3.3.10	5.2.4	Requirement					

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	1623	1623.1	Cert		Yes	No	NA	UNK	
4.22 When the second dissociation is performed, the laboratory: A) uses a second slide, or B) adds the additional volume to the original slide?	13.3.3.10	13.3.3.10 13.4.5	-	Circle one: A B					
4.23 The volume and the timing of the NaOH addition to the wells?	13.3.3.8	13.3.3.8	-	Method Procedure					
4.24 When the slides are dried (e.g., room temperature or slide warmer), the laboratory: A) uses room temperature, or B) uses 35° to 42°C, or C) follows manufacturer's instructions?	13.3.3.12	13.3.3.12	-	Circle one: A B C					
4.25 If the laboratory has more than one option specified for slide drying, are criteria included for when each option will be used?	-	-	5.3.1	Recommendation					
4.26 That positive and negative staining controls are prepared at the same time the slides are prepared?	14.1	14.1.3	-	Requirement					
4.27 Acceptable sample purification and slide preparation procedures, including issues not noted in items 4.1 through 4.26?				Critical GLP					
5 Sample Staining									
5.1 Which stain to use and to follow manufacturer's instructions for FITC stain application?	14.2	14.2	5.3.2	Method Procedure					
5.2 The slides are 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					
5.3 The working DAPI stain is prepared the day it is used?	7.7.2	7.9.2	3.19.2	Method Procedure					
5.4 The stock DAPI is stored at 1 to 10°C in the dark?	7.7.1	7.9.1	3.19.1	Method Procedure					
5.5 The volume of working DAPI applied and the incubation time?	14.6	14.6	-	Method Procedure					
5.6 The technique used to drain the excess stain from the well and to rinse the well?	14.5	14.5	-	Method Procedure					
5.7 What type and amount of mounting media used?	7.8	7.10	-	Method Procedure					

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5.8 That all the edges of the cover slip are sealed well with clear fingernail polish, unless Elvanol® is used?	14.9	14.9	-	Method Procedure					
5.9 The finished slides or slides not read immediately are 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					
5.10 Acceptable sample staining procedures, including issues not noted in items 5.1 through 5.9?				Critical GLP					
6 Microscope and Examination									
6.1 Instructions for ocular and Kohler adjustments?	10.3.4 10.3.6	10.7 10.8	3.22.10	Requirement					
6.2 That all measurements must be recorded to the nearest 0.5 micron?	15.2.2.3 15.2.3.3	15.2.2.4 15.2.3.4	3.22.5	Requirement					
6.3 Microscope cleaning procedures?	10.4	10.9	3.22.11	Requirement					
6.4 The recording of coordinates of all cysts and oocysts on the worksheet for future reference; and slide orientation on the microscope stage to standardize coordinate recording?	-	-	-	Recommendation					
6.5 The examination and acceptance of positive and negative staining controls before proceeding with examination of field samples?	15.2.1	15.2.1	5.4.6 5.4.7	Requirement					
6.6 That each analyst characterizes 3 oocysts and 3 cysts on the positive staining control at each examination session?	15.2.1.1	15.2.1.1	5.4.6	Requirement					
6.7 Corrective actions if positive and/or negative staining controls are not acceptable?	-	-	5.4.8	Recommendation					
6.8 The criteria for organism identification?	15.2.2	15.2.2 15.2.3	5.4.9 5.4.10	Requirement					
6.9 Every positive organism in a field sample is characterized and recorded?	15.2	15.2.2.1 15.2.3.1	5.4.9.1 5.4.10.1	Requirement					
6.10 Acceptable microscope and examination procedures, including issues not noted in items 6.1 through 6.9?				Requirement GLP					
7 Reagents									

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	1623	1623.1	Cert		Yes	No	NA	UNK	
7.1 Procedures for the preparation of all essential chemicals and reagents?	7.0	7.0	4.2	Critical					
7.2 That expiration dates are specified for all reagents prepared by the laboratory?	-	-	4.2.2	Critical					
8 Quality Assurance									
8.1 Training protocol for new employees?	9.1	9.1	1.7	Requirement GLP					
8.2 Procedures for performing analyst verification?	10.6	9.10	7.1.9	Requirement GLP					
8.3 Positive and interfering organisms detected in field samples are documented by photography?	-	-	5.4.11	Recommendation					
8.4 Acceptable procedures for sample collection for field or utility personnel?	-	-	6.1	Critical GLP					
8.5 Criteria for sample acceptance and corrective action procedures?	8.1.3	8.1.3	6.	Requirement GLP					
8.6 Method required holding times?	8.2	8.2	6.4	Requirement GLP					
8.7 Manual data recording procedures?	-	-	8.0	Critical GLP					
8.8 Procedures for checking the accuracy of data transcriptions, including electronic data entry?	-	-	8.1	Critical GLP					
8.9 Procedures for checking the accuracy of manual calculations?	-	-	8.1	Critical GLP					
8.10 Procedures for electronic data entry and storage?	-	-	8.2	Critical GLP					
8.11 How backup of stored data is performed?	-	-	8.2	Critical GLP					
8.12 Corrective action procedures for OPR failures?	9.7.4	9.8.5	7.1.6.2	Requirement GLP					
8.13 Corrective action procedures for method blank contamination?	9.6.2	9.7.3	7.1.5.2	Requirement GLP					

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8.14 Procedures for identifying and assessing declining trends in recovery through review of control charts and/or other recovery data?	-	-	7.1.7.2	Recommendation GLP					
8.15 Corrective action procedures for investigating QC failures or declining trends in recovery?	-	-	7.1.7.2	Recommendation GLP					
8.16 Acceptable glassware washing procedures?	-	-	4.4	Critical GLP					

Comments: