

A119 – CALA CHECKLIST FOR CRYPTOSPORIDIUM/GIARDIA

Revision #1.6

August 30, 2019

Laboratory Name: _____

Appendix Name: _____

Appendix Number: _____

Assessor: _____

Date: _____



CALA
Trust, measured accurately

ASSESSOR NOTES:

CRYPTOSPORIDIUM/GIARDIA FILTRATION CHECKLIST

Item	Clause	Requirement	Document Review 123	Implementation 123
METHOD SELECTION				
	7.2.1.2	Document review: verify that there is a documented method. Implementation: verify that the current authorized test method and necessary supporting work instructions are available to the analyst. (Based on EPA 1622/1623, December 2005.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
TEST METHOD VALIDATION/VERIFICATION				
01	7.2.1.5 or 7.2.2	Document review: verify that there are method verification (7.2.1.5) or validation (7.2.2) results, and a statement that the method is fit for the intended use. Implementation: Method is validated or verified in laboratory and includes:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Analyst Competence;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Initial Precision & Recovery (spike and process 4 reagent water samples);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Method Blank;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Matrix Spike;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Method Precision: after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery (S _r);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Participate in PT: as per P02-03 – CALA Program Description - Proficiency Testing Policy for Accreditation; lab follows up on any unsatisfactory results.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Statement of Laboratory Accuracy: calculate the mean % recovery (R) and SD % recovery (S _r). Express accuracy as a recovery interval from R-2 S _r to R+2 S _r . (Ex. if R = 95% and S _r = 25%, the accuracy is 45% to 145%).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Procedures required for method modifications:		
		• Initial Precision & Recovery (IPR);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Matrix Spike/Matrix Spike duplicates (recommended).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
METHOD SELECTION				

Item	Clause	Requirement	Document Review 123	Implementation 123
	7.2.1.3	Verify that all necessary successive steps in the test procedure are adequately documented in the test method, and are based on the latest valid edition of a published reference method, including:		
		• details on reagent preparation, storage and shelf-life;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• equipment;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• supplies;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• processing.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Test Method Procedures (Processing Flowchart helpful).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Sample Filtration and Elution (EPA 1623:12.0)	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Envirochek capsule – Filtration, Elution, Concentration;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Filta-Max – Filtration, Elution (wash station OR stomacher), Concentration (Filta-Max concentrator OR Centrifuge), Membrane Elution (manual or stomacher wash);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Portable Continuous-flow Centrifugation.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Sample Concentration and Separation (Purification)		
		• Adjustment of Pellet Volume (analyze entire (all subsamples) or partial sample);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• IMS – capture, dissociation.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Staining	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Examination		
		• FITC or DAPI or DIC	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
SAMPLE HANDLING				
	7.4	Verify that sample history requirements are 1) documented and readily available and 2) appropriate and implemented; i.e.,		

Item	Clause	Requirement	Document Review 123	Implementation 123
		Procedures specified to protect integrity of sample during transport, including:		
		• Sampling Instructions for clients;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Sample Volume: described/documented, qualify results as necessary;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Shipping Temperature Monitoring:		
		• for filters – temperature	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• sample or thermometer vial, infrared thermometer;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• for filters and bulk samples - data logger (calibrated), temperature strip;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• ice/cold packs required.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Sample Acceptance and Holding Times Criteria:		
		• sample volume;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• samples received same day or kept cool during transport (0°C < specimen temperature < 20°C) or must reject;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• moisture provided (filters).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Processing Times:		
		• samples eluted <96 hrs after filtration in field or filtered <96hrs after bulk collection;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• elution, concentration, purification, applied to slide – in one day;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• staining <72hs after application to slide;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• reading <7days after staining (adjust if fading).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
EQUIPMENT (SUPPLIES)				
	6.4	Document review: verify that procedures are in place for maintenance of oocyst and cyst stocks, and that they are documented and readily available.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Implementation: verify that procedures are followed.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Document Review 123	Implementation 123
		Oocyst and Cyst Stocks - for Staining Controls, etc.		
		<ul style="list-style-type: none"> • Crypto oocyst stock – unstained, not formalin-fixed, <i>C. parvum</i> < 3 months old – Sterling Parasitology Lab, Uof Arizona; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Giardia cyst stock – unstained, not formalin-fixed, <i>G. intestinalis</i> < 2 weeks old - Waterborne Inc New Orleans, Hyperion Research – Medicine Hat. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Oocyst and Cyst Spikes - Flow Cytometer-Counted Spiking Suspensions required.		
		<ul style="list-style-type: none"> • BioTechnology Frontiers (BTF) Easyseed; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Wisconsin State Laboratory of Hygiene. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Procedure – Preparing Spikes – reagent water spikes (IPR), matrix spikes.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
ASSURING THE VALIDITY OF RESULTS				
	7.7	Verify that method (and media) quality control is 1) either included or referenced in the test method and 2) implemented; i.e., Method QC.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Initial Demonstration of Laboratory Capability (IDC).		
	6.2	<ul style="list-style-type: none"> • <u>Analyst Competence:</u> <ul style="list-style-type: none"> • résumés, training records, number of samples 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	7.7	<ul style="list-style-type: none"> • <u>Initial Precision and Recovery (IPR):</u> <ul style="list-style-type: none"> • Spiking procedure - perform X4 with 100-500 oocysts; • One Method blank – include with the 4 spikes; • Method Modifications: separate IPR for each modification; • Criteria: >50% oocysts must be intact - characterize (FITC, DAPI, DIC) and document • Criteria: Precision - % recovery for each organism, mean percent recovery and RSD (SD/meanX100) meets IPR acceptance criteria EPA 1623:Tables 3 & 4. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Ongoing Demonstration of Laboratory Capability and Method Performance (ODC):		

Item	Clause	Requirement	Document Review 123	Implementation 123
	6.2	<ul style="list-style-type: none"> Analyst Competence: monthly verification & corrective actions 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	7.7	<ul style="list-style-type: none"> Ongoing Precision and Recovery (OPR): (EPA 1623:9.7) - one/wk or 20 samples. 		
		<ul style="list-style-type: none"> Enumerated spiking suspension in reagent water; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Method modifications - separate OPR for each modification. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Criteria: <ul style="list-style-type: none"> >50% oocysts must be intact - characterize (FITC, DAPI, DIC) and document; unacceptable, samples associated with blank are unacceptable. Halt analysis until follow up OPR is acceptable. Precision - % recovery for each organism, meets acceptance criteria EPA 1623: Tables 3 & 4. Express as %recovery interval from P-2s to P+2s for each matrix. If recovery 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Statement of Laboratory Accuracy: calculate the mean % recovery (R) and SD % recovery (Sr). Express accuracy as a recovery interval from R-2 Sr to R+2 Sr. (Ex. if R = 95% and Sr = 25%, the accuracy is 45% to 145%) 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Matrix Spike: procedure to determine number of internal spikes from each source, including 1st sampling event (preferably): <ul style="list-style-type: none"> taken from same location as field sample and ± 10% of field sample volume, split or sequential samples; perform X4 with 100-500 oocysts; Criteria: %recovery meets acceptance criteria EPA 1623:Tables 3 & 4. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Method Precision: <ul style="list-style-type: none"> after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery (Sr); 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Document Review 123	Implementation 123
		<ul style="list-style-type: none"> update regularly, stratify for all sources. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> <u>Matrix Spike Duplicate</u> – not required. 		
		<ul style="list-style-type: none"> <u>Method Blank</u>: procedure to determine number, include after change of source of reagent water: 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> best analyzed immediately after IPR and OPR and prior to samples for the week; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Criteria – negative. If any interfering organism/material – samples associated with blank are assumed contaminated. Halt analysis until follow up blank is negative. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> (Optional: field replicates for precision of sampling technique, duplicate spiked samples for precision of analysis). 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<u>Staining Controls</u> :		
		stock oocysts and cysts or prepared slides (Wisconsin State Laboratory of Hygiene) or controls included with stain;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		every stain run – all analysts reading in that stain run, characterization, documented.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<u>Control Charts</u> – for processing, reagent lots, equipment, analysts		
		<ul style="list-style-type: none"> Minimum - OPR Control Charts (% recovery vs date) 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Data includes: 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Date; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Count; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Estimated # spiked; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Analyst; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> % recovery; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Mean recovery; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> SD; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Upper/lower control limits. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

METHOD SELECTION

Item	Clause	Requirement	Document Review 123	Implementation 123
	7.2.1.2	Verify that all necessary supporting work instructions are documented and readily available e.g.:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• glassware cleaning procedures;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• sample disposal procedures;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• supporting test methods (e.g., pH);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• equipment instruction manuals;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• requisite reference texts;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• computer software related procedures (including LIMS procedures, such as data entry and approval);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• procedure for checking all manual calculations;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• disinfection/sterilization and disposal of biohazardous material.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
CONDUCT OF TESTING				
	7.2.1.3	Verify that the test procedure and all supporting work instructions are performed as documented. Process flowchart recommended (see example).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implement- tion 123
EQUIPMENT			
	6.4	Verify that all instruments required for the test procedure are available, uniquely identified, functioning properly, and safeguarded from adjustments that would invalidate results, including:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> a regularly scheduled maintenance program for each piece of equipment, where appropriate and records of service where service was required; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> availability of back up equipment or a back-up plan in case of equipment failure. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Sampling Equipment:	
		<ul style="list-style-type: none"> Coolers; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Temperature monitoring devices; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Filtration equipment if done in field. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Filtration Equipment:	
		<ul style="list-style-type: none"> Envirochek; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Filta-max; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Portable Continuous-Flow Centrifuge (PCFC); 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Pump – place on effluent side of filter to reduce contamination; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Flow meter or graduated carboy. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Elution Equipment	
		<ul style="list-style-type: none"> Envirochek - lab shaker, etc.; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Filta-max (or stomacher). 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Concentration Equipment	
		<ul style="list-style-type: none"> Centrifuge – traceable calibration, RPM and RCF (relative centrifugal force) 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> RCF = $0.0000118rN^2$ where r = rotational radius (cm) and N = rotating speed (rpm); 	
		<ul style="list-style-type: none"> Filtamax – concentrator, magnetic stirring plate, etc. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Spiking Equipment	
		<ul style="list-style-type: none"> 10L container with spigot - discard after one use - OR 10L carboy with bottom delivery port – calibrate 10L and mark level with waterproof marker OR inline spiking channel. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		IMS Apparatus	

Item	Clause	Requirement	Implement- tion 123
		• sample mixer;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• vortex;;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• magnetic particle concentrator for 10mL test tubes;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• magnetic particle concentrator for microcentrifuge tubes;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Leighton tubes	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Staining Equipment	
		• Humid chamber;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Slide warmer (optional).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Microscope - dedicate microscope to settings to assure reproducible results;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• ocular micrometers, 20X and 100X objectives, DIC, FA 450-490nm exciter filter, 51-nm beam splitting mirror, 515-520 nm barrier filter, DAPI filters, non-fluorescing immersion oil Type FF;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• light bulb log - maximum: 50 watt – 100 hrs, 100 watt – 200 hrs;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• epifluorescent mercury bulb adjustment, transmitted bulb adjustment, interpupillary adjustment, ocular adjustment;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• calibration of ocular micrometer;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Köhler illumination.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Micropipette(s) – 0-10ul, 10-100uL, 100-1000uL:	
		• traceable calibration – at least annual;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• in-house checks 10 replicates at 100/50/10% of capacity – RSD <1% & trueness<1% for each capacity.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Refrigerators for sample and reagent storage are maintained within the specified temperature range and temperatures monitored and recorded daily, no frost-free freezers.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Incubators checked annually, maintained within the specified temperature range; temperatures monitored and recorded at least once daily (suggest continuous monitoring or wice daily or using a min-max thermometer) (if used for staining).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

SUPPORT EQUIPMENT

Item	Clause	Requirement	Implement- tion 123
	6.4	Verify that all support equipment required for the test procedure is available, functioning properly, and where necessary, calibrated; e.g., computers, pH met:	
		<ul style="list-style-type: none"> • Analytical Balance – traceable calibration (0.1mg); 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Top Load Balance – traceable calibration (10mg);; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • pH meter – calibration, scale graduations 0.1 units; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Vacuum source – 25 in Hg, with gauge and shutoff valve; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Shipping temperature monitoring devices - thermometer vial, data logger, infrared thermometer – traceable calibration; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Thermometers – traceable calibration; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Timers - traceable calibration; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • Autoclave - procedures to ensure autoclave is functioning properly (e.g., monthly test of autoclave performance using a spore strip or spore suspension, capable of demonstrating a 6 log kill of <i>Bacillus stearothermophilus</i>), log of autoclave use - i.e., items, temperature, pressure, time. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implement- tion 123
OUT OF SERVICE EQUIPMENT			
	6.4.9	Verify that out of service equipment is clearly isolated or clearly labeled or marked as being out of service, and that equipment is checked and validated before return to service.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
09	EQUIPMENT REQUIRING CHECKS OR CALIBRATION		
	6.4.8	Verify that all equipment requiring checks or calibration is labeled to indicate the status, including the date last checked/calibrated and expiry date or date when due* (e.g., checks of biosafety cabinet, calibration of semi-automated pipettes and thermometers). * not required for equipment checked daily or as-used; see P07.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
SUPPLIES – AVAILABILITY			
	6.4	Verify that all supplies required for the test procedure are available and meet requisite requirements and/or specifications (includes test organisms, reagents, reference materials,); specifically:	
		• records of reference standard/material certificates:	
		• stock oocysts and cysts;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• flow cytometer-counted spiking suspensions;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• NaOH and HCL – must be unadjusted from supplier;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• solvents – acetone, glycerol, ethanol and methanol – ACS reagent grade;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• if producing water in-house and it is used to make media or reagents, check conductivity daily or as-used and verify it is analyzed for parameters as per the most current version of Standard Methods 9020;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• if purchasing distilled water, and it is used to make media or reagents, verify that total heavy metal requirements are met (see most current version of Standard Methods) and do HPC checks monthly or on each batch purchased;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• sterile rinse buffer/distilled water available.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	Sampling/Filtration/Elution		
		• Bulk Sample containers - 10L – use only once;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implement- tion 123
		<ul style="list-style-type: none"> Tubing. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Filta-max	
		<ul style="list-style-type: none"> Filta-max foam filter - check at least 1 filter per batch that it expands properly before shipping filters to the field; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Membrane filters - FMC 10800; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> conical centrifuge tubes - 50 mL, 250 mL. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Envirochek	
		<ul style="list-style-type: none"> Envirochek sampling capsule; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Conical centrifuge tubes - 250 mL conical. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		IMS	
		<ul style="list-style-type: none"> 10 mL, 1 mL graduated pipettes; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> microcentrifuge tubes - conical, graduated, 1.5mL, 50mL and 150mL; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Dynabeads or equivalent. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Staining	
		<ul style="list-style-type: none"> Direct antibody labeling reagents - MeriFluor, Aqua-Glo, Crypt-a-Glo/Giardia-a-Glo, or EasyStain; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> If using multiple types, demonstrate performance (precision and recovery) for each time and +/- controls for each batch; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Monitor for each source water type; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Mounting medium DABCO, MeriFluor, Aqual-Glo, EasyStain, Elvanol or equivalent permanent, non-fade archiving mounting medium. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implement- tion 123
SUPPLIES- STORAGE			
	6.4	Verify that all supplies are stored under appropriate conditions (as specified in reference method or by regulator etc.) and in a manner which satisfies requirements for safety, security, separation of incompatible materials, and ease of retrieval.	
		Reagents	
		• Eluting Buffers - 1 week or until turbid;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Laureth 12 - 10% solution in reagent water, 10mL aliquots, room temp 2 months, frozen 1 yr;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Reagents for IMS - as per manufacturer;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Antibody labeling reagents and diluent (PBS) - 1o-10o C, dark. Discard diluted reagent after 48 hrs or expiry date;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• DAPI - stock solution – 1o -10o C, dark, discard when (+) control fails or after time determined by lab;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• DAPI - staining solution - prepare daily, 1o -10o C, dark. DAPI concentration may be increased if fading but solution must be tested first on environmental samples to confirm that staining intensity is appropriate.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
SUPPLIES- LABELING			
	6.4.8	Verify that all reagents and media (above) are labeled with material, concentration or purity, date prepared and/or expiry date; verify that media is appropriately labeled, stored under proper conditions, and storage times are met.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
SUPPLIES – TEST ORGANISM ID			
	6.4.8	Verify that all information required to properly identify test organisms appears on their containers (i.e., name or number of organism, and date subcultured).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implement- tion 123
SUPPLIES - LABWARE			
	6.4	Verify that all labware is adequately cleaned and, where required, labware quality control incorporates analytical testing; specifically:	
		<ul style="list-style-type: none"> • use of clean labware 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
RECORD KEEPING			
	7.5	Maintain records related to the performance of the test method; e.g.:	
		<ul style="list-style-type: none"> • analyst worksheet or notebook 1 – microscope log book (stain controls), bench sheets, slide examination forms. Include content of EPA microscope log book, bench sheets, slide examination forms as appropriate; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • record of nonconformances and actions taken 2- corrective actions for OPR failures, method blank contamination, staining control failures; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • reagent preparation log³; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • equipment maintenance log⁴; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • stock culture maintenance logs; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of gravimetric traceability⁶; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of volumetric traceability⁷; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of temperature traceability⁸; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of environmental conditions monitored; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records to define the quality of data generated. (Laboratory Accuracy Statements); 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of analyst training and competency; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> • records of sample receipt information – date/time of sampling & receipt, sample condition, transportation. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
ACCOMMODATION AND ENVIRONMENTAL CONDITIONS			

Item	Clause	Requirement	Implement- tion 123
	6.3	Verify that environmental conditions do not adversely affect the quality of any measurement:	
		• effective separation between incompatible activities;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• appropriate surfaces (smooth surface on floors, walls, ceiling and benches;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• access to laboratory controlled (cite B.02.06);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• good housekeeping;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• disinfectants available and used routinely for cleaning bench area.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
ANALYST COMPETENCE			
	6.2	Person responsible for signing authority and data validation possesses the technical knowledge relevant to the scope of accreditation (cite B.01.01).	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Verify that technicians have demonstrated competency relative to the test being accredited. Note: There is no standard reference material (slides) available (i.e. enumerated DAPI (+/-) oocysts).	
		• analyst – if astigmatism, wear glasses or contact lenses;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• training procedure and records;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• monthly verification procedure and records.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Single/Multiple Analysts:	
		• Maintain Protozoa library - photographs (FA, DAPI, DIC) and diagrams of oocysts and interfering materials, describe, quantify;	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Monthly/as used – prepare slide with 40-200 cysts and 40-200 oocysts with >50% positive DAPI and undamaged under DIC:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Each analyst counts and records total undamaged oocysts by FITC. Counts must be ≤10% of each other. If fail, identify source of variability and repeat verification.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• On same slide or any OPR, MS or (+) stain control slide, select 10 oocysts and 10 cysts:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		• Each analyst determines and records:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		▪ DAPI category – DAPI (-), DAPI (+), DAPI (+ - number of nuclei);	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		▪ DIC category – empty, containing amorphous structures, containing identifiable internal structures.	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Implementation 123
		<ul style="list-style-type: none"> Discuss and resolve differences among analysts; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> Document verification (names, date, results, pass/fail, results of attempts, corrective actions) 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		Single Analyst	
		<ul style="list-style-type: none"> Perform repetitive counts of a single verification FITC slide. 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
REPORTS			
	7.8	Verify that test report content is complete:	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> appropriate reporting of non-detects, taking dilution factors and sample volumes into consideration; 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
		<ul style="list-style-type: none"> procedures in place for reporting of adverse results to authorities having jurisdiction 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

1 includes, as appropriate, calibration data, test date (including QC data), experimental variables (e.g. temperature, etc.); analyst ID; sample ID; equipment ID; test organism lot no; test method ID; date and time of test.

2 includes as appropriate, nonconformances related to: test method variances; sample history; method performance; interferences; and data validation.

3 includes, as appropriate, supplier, grade, batch no; dates of preparation of verification; measurement of weights, volumes, time intervals, temperatures and related calculations; relevant processes (e.g., pH adjustment, sterilization); verification results; discard date.

4 includes, as appropriate, identity of the equipment and its software; manufacturer, model, serial no; checks that equipment complies with laboratory specifications; date commissioned; repair and maintenance history; calibration history; any damage, malfunction or modification to the equipment; location.

5 includes, as appropriate, organism name; date of subculture and initial of technician; purity check on non-selective medium each time the working subculture is transferred (generally, this is done weekly)

6 includes, as appropriate, traceability of balance and/or weights to a national standard and daily or as-used checks. (See A61-01 CALA Traceability Policy)

7 includes, as appropriate, traceability of auto pipettes, dilutors, etc. that play a defining role in analytical accuracy, and daily or as-used checks (see A61-01 CALA Traceability Policy).

8 includes, as appropriate, traceability of working thermometers to a national standard for those working thermometers that measure temperatures that play a defining role in analytical uncertainties (see A61-01 – CALA Traceability Policy).