

A119 – CALA Checklist for Cryptosporidium/Giardia Revision 1.4 – February 2011

Laboratory Name: _____

Appendix Name: _____

Appendix Number: _____

Assessor: _____

Date: _____



CALA
Laboratory Accreditation

Please record the following information related to this appendix:

A. Equipment I.D.

	Manufacturer	Model No.
Auto Sampler (if applicable)	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
Equipment/Instrument(s) used in analysis	_____	_____
_____	_____	_____
_____	_____	_____
Software used for data collection (including version number), if applicable	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

B. Proficiency Testing Requirements

Please review Proficiency Testing (PT) information on the cover sheet to the appendix. Make any changes or additions directly on the cover sheet. For requirements on options and number of analytes that must have PT, refer to P02-03 - *Program Description - Proficiency Testing Policy for Accreditation*.

C. Analyst I.D. (Section 5.2 of ISO/IEC 17025)

	Primary Analyst	Back-up Analyst
Name	_____	_____
Position	_____	_____
Degree/Diploma	_____	_____
Years Analytical Experience*	_____	_____
Analyst Proficiency**	_____	_____
Check if interviewed	_____	_____

* Years of analytical experience related to the appendix being assessed.

** Please record the date that the analyst was deemed competent to perform the appendix OR the date that he/she last successfully participated in Proficiency Testing (PT).

Cryptosporidium/Giardia Filtration Checklist

Item	Clause	Requirement	Document Review			Implementation		
			1	2	3	1	2	3
01		DOCUMENT CONTROL						
	4.3	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"/>
02		TEST METHOD VALIDATION/VERIFICATION						
01	5.4	<p>Document review: verify that there are method validation results, and a statement that the method is fit for the intended use.</p> <p>Implementation: Method is validated or verified in laboratory and includes:</p> <ul style="list-style-type: none"> ▪ Analyst Competence; ▪ Initial Precision & Recovery (spike and process 4 reagent water samples); ▪ Method Blank; ▪ Matrix Spike; ▪ <u>Method Precision:</u> after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery (S_r) - (See 06); ▪ <u>Successfully participate in PT:</u> as per P02-03 - <i>CALA Program Description - Proficiency Testing Policy for Accreditation</i> ▪ <u>Statement of Laboratory Accuracy:</u> 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%). <p>Note: For records of Method Validation, cite B.03.09 in A02.</p> <p>Procedures required for method modifications:</p> <ul style="list-style-type: none"> ▪ Initial Precision & Recovery (IPR); ▪ Matrix Spike/Matrix Spike duplicates (recommended). 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Item	Clause	Requirement	Document Review			Implementation		
			1	2	3	1	2	3
05		TEST METHOD - OOCYST AND CYST STOCKS						
01	5.4.1 5.4.2	<p>Document review: verify that procedures are in place for maintenance of oocyst and cyst stocks, and that they are documented and readily available.</p> <p>Implementation: verify that procedures are followed.</p> <p>Oocyst and Cyst Stocks - for Staining Controls, etc.</p> <ul style="list-style-type: none"> ▪ Crypto oocyst stock - unstained, not formalin-fixed, C. parvum < 3 months old - Sterling Parasitology Lab, Uof Arizona; ▪ Giardia cyst stock - unstained, not formalin-fixed, G. intestinalis < 2 weeks old - Waterborne Inc New Orleans, Hyperion Research - Medicine Hat. <p>Oocyst and Cyst Spikes - Flow Cytometer-Counted Suspensions required.</p> <ul style="list-style-type: none"> ▪ BioTechnology Frontiers (BTF) Easyseed; ▪ Wisconsin State Laboratory of Hygiene. <p>Procedure - Preparing Spikes - reagent water spikes (IPR), matrix spikes.</p>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>				
06		METHOD QUALITY CONTROL						
01	5.9	<p>Verify that method (and media) quality control is 1) either included or referenced in the test method and 2) implemented; i.e., Method QC.</p> <p>Initial Demonstration of Laboratory Capability (IDC).</p> <ul style="list-style-type: none"> ▪ <u>Analyst Competence:</u> <ul style="list-style-type: none"> • résumés, training records, number of samples (See Checklist 13-01). 	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>				

Item	Clause	Requirement	Document Review			Implementation												
			1	2	3	1	2	3										
METHOD QUALITY CONTROL (continued)																		
01	5.9	<ul style="list-style-type: none"> ▪ 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 ▪ <u>Statement of Laboratory Accuracy</u>: 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%) ▪ <u>Matrix Spike</u>: 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. ▪ <u>Method Precision</u>: <ul style="list-style-type: none"> • after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery (S_r); • update regularly, stratify for all sources. ▪ <u>Matrix Spike Duplicate</u> - not required. ▪ <u>Method Blank</u>: procedure to determine number, include after change of source of reagent water: <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"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Item	Clause	Requirement	Document Review			Implementation		
			1	2	3	1	2	3
07		TEST METHOD CONTENT-OTHER WORK INSTRUCTIONS (PROCEDURES)						
01	5.4.1	Verify that all necessary supporting work instructions are documented and readily available e.g.: <ul style="list-style-type: none"> ▪ glassware cleaning procedures; ▪ sample disposal procedures; ▪ supporting test methods (e.g., pH); ▪ equipment instruction manuals; ▪ requisite reference texts; ▪ computer software related procedures (including LIMS procedures, such as data entry and approval); ▪ procedure for checking all manual calculations; ▪ 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"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
08		CONDUCT OF TESTING						
01	5.4.1 4.2.1	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	1	2	3
09		EQUIPMENT (continued)			
01	5.5.1 5.5.2 5.5.4 5.5.12	<p>Staining Equipment</p> <ul style="list-style-type: none"> ▪ Humid chamber; ▪ Slide warmer (optional). <p>Microscope - dedicate microscope to settings to assure reproducible results;</p> <ul style="list-style-type: none"> ▪ 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; ▪ light bulb log - maximum: 50 watt - 100 hrs, 100 watt - 200 hrs; ▪ epifluorescent mercury bulb adjustment, transmitted bulb adjustment, interpupillary adjustment, ocular adjustment; ▪ calibration of ocular micrometer; ▪ Köhler illumination. <p>Micropipette(s) - 0-10uL, 10-100uL, 100-1000uL:</p> <ul style="list-style-type: none"> ▪ traceable calibration - at least annual; ▪ in-house checks 10 replicates at 100/50/10% of capacity - RSD <1% & trueness<1% for each capacity. <p>Refrigerators for sample and reagent storage are maintained within the specified temperature range and temperatures monitored and recorded daily, no frost-free freezers.</p> <p>Incubators checked annually, maintained within the specified temperature range; temperatures monitored and recorded at least once daily (suggest continuous monitoring or twice daily or using a min-max thermometer) (if used for staining).</p>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
09		SUPPORT EQUIPMENT			
02	5.5.2	<p>Verify that all support equipment required for the test procedure is available, functioning properly, and where necessary, calibrated; e.g., computers, pH met:</p> <ul style="list-style-type: none"> ▪ Analytical Balance - traceable calibration (0.1mg); ▪ Top Load Balance - traceable calibration (10mg);; ▪ pH meter - calibration, scale graduations 0.1 units; ▪ Vacuum source - 25 in Hg, with gauge and shutoff valve; ▪ Shipping temperature monitoring devices - thermometer vial, data logger, infrared thermometer - traceable calibration; ▪ Thermometers - traceable calibration; ▪ Timers - traceable calibration; ▪ 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 (cite 07.01). 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Item	Clause	Requirement	1	2	3
10		SUPPLIES - AVAILABILITY (continued)			
01	4.6.2 5.5.1	<p>Envirochek</p> <ul style="list-style-type: none"> ▪ Envirochek sampling capsule; ▪ Conical centrifuge tubes - 250 mL conical. <p>IMS</p> <ul style="list-style-type: none"> ▪ 10 mL, 1 mL graduated pipettes; ▪ microcentrifuge tubes - conical, graduated, 1.5mL, 50mL and 150mL; ▪ Dynabeads or equivalent. <p>Staining</p> <ul style="list-style-type: none"> ▪ Direct antibody labeling reagents - MeriFluor, Aqua-Glo, Crypt-a-Glo/Giardia-a-Glo, or EasyStain; ▪ If using multiple types, demonstrate performance (precision and recovery) for each time and +/- controls for each batch; ▪ Monitor for each source water type; ▪ 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"/>
10		SUPPLIES- STORAGE			
02	5.3	<p>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.</p> <p>Reagents</p> <ul style="list-style-type: none"> ▪ Eluting Buffers - 1 week or until turbid; ▪ Laureth 12 - 10% solution in reagent water, 10mL aliquots, room temp 2 months, frozen 1 yr; ▪ Reagents for IMS - as per manufacturer; ▪ Antibody labeling reagents and diluent (PBS) - 1°-10° C, dark. Discard diluted reagent after 48 hrs or expiry date; ▪ DAPI - stock solution - 1° -10° C, dark, discard when (+) control fails or after time determined by lab; ▪ DAPI - staining solution - prepare daily, 1° -10° 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"/>
10		SUPPLIES- LABELING			
03	4.13.2	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"/>

Item	Clause	Requirement	1	2	3
10		SUPPLIES - TEST ORGANISM ID			
04	4.13.2	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"/>
10		SUPPLIES - LABWARE			
05	5.5.1	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"/>
11		RECORD KEEPING			
01		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; ▪ record of nonconformances and actions taken (2) - corrective actions for OPR failures, method blank contamination, staining control failures; ▪ reagent preparation log (3) See 03-1; ▪ equipment maintenance log (4) See 09-1; ▪ stock culture maintenance log (5) See 05-1; ▪ records of gravimetric traceability (6) See 09-1; ▪ records of volumetric traceability (7) See 09-1; ▪ records of temperature traceability (8) See 09-1; ▪ records of environmental conditions monitored See 12-01; ▪ records to define the quality of data generated. (Laboratory Accuracy Statements) (See 02-01); ▪ records of analyst training and competency See 13-01; ▪ records of sample receipt information - date/time of sampling & receipt, sample condition, transportation. 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12		ACCOMMODATION AND ENVIRONMENTAL CONDITIONS			
01	5.3.1 5.3.2 5.3.3	Verify that environmental conditions do not adversely affect the quality of any measurement: <ul style="list-style-type: none"> ▪ effective separation between incompatible activities (cite B.02.05); ▪ appropriate surfaces (smooth surface on floors, walls, ceiling and benches (cite B.02.01)); ▪ access to laboratory controlled (cite B.02.06); ▪ good housekeeping(cite B.02.07); ▪ disinfectants available and used routinely for cleaning bench area. 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Item	Clause	Requirement	1	2	3
13		ANALYST COMPETENCE			
01		<p>Person responsible for signing authority and data validation possesses the technical knowledge relevant to the scope of accreditation (cite B.01.01).</p> <p>Verify that technicians have demonstrated competency relative to the test being accredited (cite B.01.01).</p> <p>Note: There is no standard reference material (slides) available (i.e. enumerated DAPI (+/-) oocysts).</p> <ul style="list-style-type: none"> ▪ analyst - if astigmatism, wear glasses or contact lenses; ▪ training procedure and records; ▪ monthly verification procedure and records. <p>Single/Multiple Analysts:</p> <ul style="list-style-type: none"> ▪ Maintain Protozoa library - photographs (FA, DAPI, DIC) and diagrams of oocysts and interfering materials, describe, quantify; ▪ Monthly/as used - prepare slide with 40-200 cysts and 40-200 oocysts with >50% positive DAPI and undamaged under DIC: <ul style="list-style-type: none"> • Each analyst count and record total undamaged oocysts by FITC. Counts must be ≤10% of each other. If fail, identify source of variability and repeat verification. ▪ On same slide or any OPR, MS or (+) stain control slide, select 10 oocysts and 10 cysts: <ul style="list-style-type: none"> • Each analyst determine and record: <ul style="list-style-type: none"> • DAPI category - DAPI (-), DAPI (+), DAPI (+ - number of nuclei); • DIC category - empty, containing amorphous structures, containing identifiable internal structures. • Discuss and resolve differences among analysts; ▪ Document verification (names, date, results, pass/fail, results of attempts, corrective actions) <p>Single Analyst</p> <ul style="list-style-type: none"> ▪ Perform repetitive counts of a single verification FITC slide. 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14		REPORTS			
		<p>Verify that test report content is complete:</p> <ul style="list-style-type: none"> ▪ appropriate reporting of non-detects, taking dilution factors and sample volumes into consideration (cite B.09.02); ▪ procedures in place for reporting of adverse results to authorities having jurisdiction (cite A.03.01). 	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Footnotes for Record-Keeping (Section 11 01, above):

- (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- 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-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 - CALA Traceability Policy).