# A119 – CALA Checklist for Cryptosporidium/Giardia
**Revision 1.5** – December 12, 2014

**Laboratory Name:** _____

**Appendix Name:** _____

**Appendix Number:** _____

**Assessor:** _____

**Date:** _____

CALA
Laboratory Accreditation

# Assessor Notes:

# Cryptosporidium/Giardia Filtration Checklist

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 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.) | ❑ ❑ ❑ | ❑ ❑ ❑ |
| **02** | | **TEST METHOD VALIDATION/VERIFICATION** | | |
| 01 | 5.4 | **Document review**: verify that there are method validation results, and a statement that the method is fit for the intended use.<br><br>**Implementation**: Method is validated or verified in laboratory and includes: | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Analyst Competence; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Initial Precision & Recovery (spike and process 4 reagent water samples); | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Method Blank; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Matrix Spike; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Method Precision: after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery ($S_r$) - (See 06).; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Participate in PT: as per P02-03 – *CALA Program Description - Proficiency Testing Policy for Accreditation; lab follows up on any unsatisfactory results.* | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ 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%). | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Note**: For records of Method Validation, cite B.03.09 in A02. | | |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| | | **Procedures required for method modifications**:<br>▪ Initial Precision & Recovery (IPR); | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Matrix Spike/Matrix Spike duplicates (recommended). | ❑ ❑ ❑ | ❑ ❑ ❑ |
| **03** | | **TEST METHOD** | | |
| 01 | 4.2.1<br>5.4.1 | 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; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ equipment; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ supplies; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ processing. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Test Method Procedures** (Processing Flowchart helpful). | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Sample Filtration and Elution** (EPA 1623:12.0) | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Envirochek capsule – Filtration, Elution, Concentration; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Filta-Max – Filtration, Elution (wash station OR stomacher), Concentration (Filta-Max concentrator OR Centrifuge), Membrane Elution (manual or stomacher wash); | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Portable Continuous-flow Centrifugation. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Sample Concentration and Separation** (Purification) | | |
| | | ▪ Adjustment of Pellet Volume (analyze entire (all subsamples) or partial sample); | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ IMS – capture, dissociation. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Staining** | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Examination**<br>▪ FITC or DAPI or DIC | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| **04** | | **SAMPLING** | | |
| 01 | 5.7 | Verify that sample history requirements are 1) documented and readily available and 2) appropriate and implemented; i.e., | | |
| | | Procedures specified to protect integrity of sample during transport, including: | | |
| | | ▪ Sampling Instructions for clients; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Sample Volume: described/documented, qualify results as necessary; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Shipping Temperature Monitoring: | | |
| | | • for filters – temperature | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • sample or thermometer vial, infrared thermometer; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • for filters and bulk samples - data logger (calibrated), temperature strip; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • ice/cold packs required. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Sample Acceptance and Holding Times Criteria: | | |
| | | • sample volume; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • samples received same day or kept cool during transport ($0^{\circ}C$ < specimen temperature < $20^{\circ}C$) or must reject; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • moisture provided (filters). | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Processing Times: | | |
| | | • samples eluted <96 hrs after filtration in field or filtered <96hrs after bulk collection; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • elution, concentration, purification, applied to slide – in one day; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • staining <72hs after application to slide; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • reading <7days after staining (adjust if fading). | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| **05** | | **TEST METHOD – OOCYST AND CYST STOCKS** | | |
| 01 | 5.4.1<br>5.4.2 | **Document review**: verify that procedures are in place for maintenance of oocyst and cyst stocks, and that they are documented and readily available. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Implementation**: verify that procedures are followed. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Oocyst and Cyst Stocks** - for Staining Controls, etc. | | |
| | | ▪ 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. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Oocyst and Cyst Spikes -** Flow Cytometer-Counted Spiking Suspensions required. | | |
| | | ▪ BioTechnology Frontiers (BTF) Easyseed; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Wisconsin State Laboratory of Hygiene. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Procedure** – Preparing Spikes – reagent water spikes (IPR), matrix spikes. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| **06** | | **METHOD QUALITY CONTROL** | | |
| 01 | 5.9 | Verify that method (and media) quality control is 1) either included or referenced in the test method and 2) implemented; i.e., Method QC. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Initial Demonstration of Laboratory Capability (IDC).** | | |
| | | ▪ Analyst Competence:<br>• résumés, training records, number of samples (See Checklist 13-01). | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| **06** | | **METHOD QUALITY CONTROL (continued)** | | |
| 01 | 5.9 | ▪ Initial Precision and Recovery **(IPR):** | | |
| | | • Spiking procedure - perform X4 with 100-500 oocysts; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • One Method blank – included 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. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **Ongoing Demonstration of Laboratory Capability and Method Performance (ODC):** | | |
| | | ▪ Analyst Competence: monthly verification & corrective actions (See Checklist 13-01) | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Ongoing Precision and Recovery (OPR): (EPA 1623:9.7) - one/wk or 20 samples. | | |
| | | • Enumerated spiking suspension in reagent water; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • Method modifications - separate OPR for each modification. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • Criteria: | | |
| | | ▪ >50% oocysts must be intact - characterize (FITC, DAPI, DIC) and document; | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| | | ▪ unacceptable, samples associated with blank are unacceptable. Halt analysis until follow up OPR is acceptable. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | **METHOD QUALITY CONTROL (continued)** | | |
| 01 | 5.9 | ▪ 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 | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ 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%) | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Matrix Spike: procedure to determine number of internal spikes from each source, including 1st sampling event (preferably): | | |
| | | • 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. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Method Precision: | | |
| | | • after 5 Matrix Spike samples, calculate mean % recovery (P) and SD% recovery ($S_r$); | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • update regularly, stratify for all sources. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Matrix Spike Duplicate – not required. | | |
| | | ▪ Method Blank: procedure to determine number, include after change of source of reagent water: | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 1 2 3 |
|---|---|---|---|---|
| | | • best analyzed immediately after IPR and OPR and prior to samples for the week; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| **06** | | **METHOD QUALITY CONTROL (continued)** | | |
| 01 | 5.9 | • Criteria – negative. If any interfering organism/material – samples associated with blank are assumed contaminated. Halt analysis until follow up blank is negative. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • (Optional: field replicates for precision of sampling technique, duplicate spiked samples for precision of analysis). | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Staining Controls: | | |
| | | • stock oocysts and cysts or prepared slides (Wisconsin State Laboratory of Hygiene) or controls included with stain; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • every stain run – all analysts reading in that stain run, characterization, documented. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ Control Charts – for processing, reagent lots, equipment, analysts | | |
| | | • Minimum - OPR Control Charts (% recovery vs date) | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • Data includes: | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • Date; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • Count; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • estimated # spiked; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • analyst; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • % recovery; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • mean recovery; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • SD; | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | • upper/lower control limits. | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | Document Review 1 2 3 | Implementation 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.: | ❑ ❑ ❑ | ❑ ❑ ❑ |
| | | ▪ 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. | ❑ ❑ ❑ | ❑ ❑ ❑ |
| 08 | | **CONDUCT OF TESTING** | | |
| 01 | 5.4.1<br>4.2.1 | Verify that the test procedure and all supporting work instructions are performed as documented.<br><br>Process flowchart recommended (see example). | ❑ ❑ ❑ | ❑ ❑ ❑ |

| Item | Clause | Requirement | 1 2 3 |
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| **09** | | **EQUIPMENT** | |
| 01 | 5.5.1<br>5.5.2<br>5.5.4<br>5.5.12 | Verify that all instruments required for the test procedure are available, uniquely identified, functioning properly, and safeguarded from adjustments that would invalidate results, including: | ❑ ❑ ❑ |
| | | ▪ a regularly scheduled maintenance program for each piece of equipment, where appropriate and records of service where service was required (cite B.04.02); | ❑ ❑ ❑ |
| | | ▪ availability of back up equipment or a back up plan in case of equipment failure (cite B.04.01). | ❑ ❑ ❑ |
| | | **Sampling Equipment:** | |
| | | ▪ Coolers; | ❑ ❑ ❑ |
| | | ▪ Temperature monitoring devices; | ❑ ❑ ❑ |
| | | ▪ Filtration equipment if done in field. | ❑ ❑ ❑ |
| | | **Filtration Equipment:** | |
| | | ▪ Envirochek; | ❑ ❑ ❑ |
| | | ▪ Filta-max; | ❑ ❑ ❑ |
| | | ▪ Portable Continuous-Flow Centrifuge (PCFC); | ❑ ❑ ❑ |
| | | ▪ Pump – place on effluent side of filter to reduce contamination; | ❑ ❑ ❑ |
| | | ▪ Flow meter or graduated carboy. | ❑ ❑ ❑ |
| | | **Elution Equipment** | |
| | | ▪ Envirochek - lab shaker, etc.; | ❑ ❑ ❑ |
| | | ▪ Filta-max (or stomacher). | ❑ ❑ ❑ |
| | | **Concentration Equipment** | |
| | | ▪ Centrifuge – traceable calibration, RPM and RCF (relative centrifugal force)<br>RCF = 0.0000118rN2 where r = rotational radius (cm) and N = rotating speed (rpm); | ❑ ❑ ❑ |
| | | ▪ Filtamax – concentrator, magnetic stirring plate, etc. | ❑ ❑ ❑ |
| | | **Spiking Equipment** | |
| | | ▪ 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. | ❑ ❑ ❑ |
| | | **IMS Apparatus** | |
| | | ▪ sample mixer; | ❑ ❑ ❑ |
| | | ▪ vortex;; | ❑ ❑ ❑ |
| | | ▪ magnetic particle concentrator for 10mL test tubes; | ❑ ❑ ❑ |
| | | ▪ magnetic particle concentrator for microcentrifuge tubes; | ❑ ❑ ❑ |
| | | ▪ Leighton tubes | ❑ ❑ ❑ |

| Item | Clause | Requirement | 1 2 3 |
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| **09** | | **EQUIPMENT (continued)** | |
| 01 | 5.5.1<br>5.5.2<br>5.5.4<br>5.5.12 | **Staining Equipment**<br>▪ Humid chamber;<br>▪ Slide warmer (optional).<br>**Microscope -** dedicate microscope to settings to assure reproducible results;<br>▪ 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;<br>▪ light bulb log - maximum: 50 watt – 100 hrs, 100 watt – 200 hrs;<br>▪ epifluorescent mercury bulb adjustment, transmitted bulb adjustment, interpupillary adjustment, ocular adjustment;<br>▪ calibration of ocular micrometer;<br>▪ Köhler illumination.<br>**Micropipette(s)** – 0-10ul, 10-100uL, 100-1000uL:<br>▪ traceable calibration – at least annual;<br>▪ in-house checks 10 replicates at 100/50/10% of capacity – RSD <1% & trueness<1% for each capacity.<br>**Refrigerators** for sample and reagent storage are maintained within the specified temperature range and temperatures monitored and recorded daily, no frost-free freezers.<br>**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). | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |
| **09** | | **SUPPORT EQUIPMENT** | |
| 02 | 5.5.2 | Verify that all support equipment required for the test procedure is available, functioning properly, and where necessary, calibrated; e.g., computers, pH met:<br>▪ **Analytical Balance** – traceable calibration (0.1mg);<br>▪ **Top Load Balance** – traceable calibration (10mg);;<br>▪ **pH meter** – calibration, scale graduations 0.1 units;<br>▪ **Vacuum source** – 25 in Hg, with gauge and shutoff valve;<br>▪ **Shipping temperature monitoring devices** - thermometer vial, data logger, infrared thermometer – traceable calibration;<br>▪ **Thermometers** – traceable calibration;<br>▪ **Timers** - traceable calibration;<br>▪ **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 Bacillus | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |

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| | | *stearothermophilus*), log of autoclave use - i.e., items, temperature, pressure, time (cite 07.01). | |
| **09** | | **OUT OF SERVICE EQUIPMENT** | |
| 03 | 5.5.7<br>5.5.9 | 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. | ❑ ❑ ❑ |
| **09** | | **EQUIPMENT REQUIRING CHECKS OR CALIBRATION** | |
| 04 | 5.5.2<br>5.5.5<br>5.5.8 | 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).<br>* not required for equipment checked daily or as-used; see P07. | ❑ ❑ ❑ |
| **10** | | **SUPPLIES – AVAILABILITY** | |
| 01 | 4.6.2<br>5.5.1 | 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:<br>• stock oocysts and cysts; | ❑ ❑ ❑ |
| | | • flow cytometer-counted spiking suspensions; | ❑ ❑ ❑ |
| | | ▪ NaOH and HCL – must be unadjusted from supplier; | ❑ ❑ ❑ |
| | | ▪ solvents – acetone, glycerol, ethanol and methanol – ACS reagent grade; | ❑ ❑ ❑ |
| | | ▪ 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; | ❑ ❑ ❑ |
| | | ▪ 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; | ❑ ❑ ❑ |
| | | ▪ sterile rinse buffer/distilled water available. | ❑ ❑ ❑ |
| | | **Sampling/Filtration/Elution**<br>▪ Bulk Sample containers - 10L – use only once; | ❑ ❑ ❑ |
| | | ▪ Tubing. | ❑ ❑ ❑ |
| | | **Filta-max**<br>▪ Filta-max foam filter - check at least 1 filter per batch that it expands properly before shipping filters to the field; | ❑ ❑ ❑ |
| | | ▪ Membrane filters - FMC 10800; | ❑ ❑ ❑ |
| | | ▪ conical centrifuge tubes - 50 mL, 250 mL. | ❑ ❑ ❑ |

| Item | Clause | Requirement | 1 2 3 |
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| **10** | | **SUPPLIES – AVAILABILITY (continued)** | |
| 01 | 4.6.2<br>5.5.1 | **Envirochek**<br>▪ Envirochek sampling capsule;<br>▪ Conical centrifuge tubes - 250 mL conical.<br>**IMS**<br>▪ 10 mL, 1 mL graduated pipettes;<br>▪ microcentrifuge tubes - conical, graduated, 1.5mL, 50mL and 150mL;<br>▪ Dynabeads or equivalent.<br>**Staining**<br>▪ Direct antibody labeling reagents - MeriFluor, Aqua-Glo, Crypt-a-Glo/Giardia-a-Glo, or EasyStain;<br>▪ If using multiple types, demonstrate performance (precision and recovery) for each time and +/- controls for each batch;<br>▪ Monitor for each source water type;<br>▪ Mounting medium DABCO, MeriFluor, Aqual-Glo, EasyStain, Elvanol or equivalent permanent, non-fade archiving mounting medium. | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |
| **10** | | **SUPPLIES- STORAGE** | |
| 02 | 5.3 | 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.<br>**Reagents**<br>▪ Eluting Buffers - 1 week or until turbid;<br>▪ Laureth 12 - 10% solution in reagent water, 10mL aliquots, room temp 2 months, frozen 1 yr;<br>▪ Reagents for IMS - as per manufacturer;<br>▪ Antibody labeling reagents and diluent (PBS) - $1^{o}$-$10^{o}$ C, dark. Discard diluted reagent after 48 hrs or expiry date;<br>▪ DAPI - stock solution – $1^{o}$ -$10^{o}$ C, dark, discard when (+) control fails or after time determined by lab;<br>▪ DAPI - staining solution - prepare daily, $1^{o}$ -$10^{o}$ 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. | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |
| **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. | ❑ ❑ ❑ |

| 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). | ❑ ❑ ❑ |
| **10** | | **SUPPLIES - LABWARE** | |
| 05 | 5.5.1 | Verify that all labware is adequately cleaned and, where required, labware quality control incorporates analytical testing; specifically:<br>▪ use of clean labware | ❑ ❑ ❑ |
| **11** | | **RECORD KEEPING** | |
| 01 | | Maintain records related to the performance of the test method; e.g.:<br>▪ 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;<br>▪ record of nonconformances and actions taken (2) - corrective actions for OPR failures, method blank contamination, staining control failures;<br>▪ reagent preparation log (3) See 03-1;<br>▪ equipment maintenance log (4) See 09-1;<br>▪ stock culture maintenance log (5) See 05-1;<br>▪ records of gravimetric traceability (6) See 09-1;<br>▪ records of volumetric traceability (7) See 09-1;<br>▪ records of temperature traceability (8) See 09-1;<br>▪ records of environmental conditions monitored See 12-01;<br>▪ records to define the quality of data generated. (Laboratory Accuracy Statements) (See 02-01);<br>▪ records of analyst training and competency See 13-01;<br>▪ records of sample receipt information – date/time of sampling & receipt, sample condition, transportation. | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |
| **12** | | **ACCOMMODATION AND ENVIRONMENTAL CONDITIONS** | |
| 01 | 5.3.1<br>5.3.2<br>5.3.3 | Verify that environmental conditions do not adversely affect the quality of any measurement:<br>▪ effective separation between incompatible activities (cite B.02.05);<br>▪ appropriate surfaces (smooth surface on floors, walls, ceiling and benches (cite B.02.01));<br>▪ access to laboratory controlled (cite B.02.06); | ❑ ❑ ❑<br>❑ ❑ ❑<br>❑ ❑ ❑ |

| Item | Clause | Requirement | 1 2 3 |
|---|---|---|---|
| | | ▪ good housekeeping(cite B.02.07); | ❑ ❑ ❑ |
| | | ▪ disinfectants available and used routinely for cleaning bench area. | ❑ ❑ ❑ |
| **13** | | **ANALYST COMPETENCE** | |
| 01 | | Person responsible for signing authority and data validation possesses the technical knowledge relevant to the scope of accreditation (cite B.01.01). | ❑ ❑ ❑ |
| | | Verify that technicians have demonstrated competency relative to the test being accredited (cite B.01.01). | |
| | | Note: There is no standard reference material (slides) available (i.e. enumerated DAPI (+/-) oocysts). | |
| | | ▪ analyst – if astigmatism, wear glasses or contact lenses; | ❑ ❑ ❑ |
| | | ▪ training procedure and records; | ❑ ❑ ❑ |
| | | ▪ monthly verification procedure and records. | ❑ ❑ ❑ |
| | | **Single/Multiple Analysts:** | |
| | | ▪ 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: | ❑ ❑ ❑ |
| | | • 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: | ❑ ❑ ❑ |
| | | • Each analyst determine and record: | ❑ ❑ ❑ |
| | | • 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) | ❑ ❑ ❑ |
| | | **Single Analyst** | |
| | | ▪ Perform repetitive counts of a single verification FITC slide. | ❑ ❑ ❑ |
| **14** | | **REPORTS** | |
| | | Verify that test report content is complete: | ❑ ❑ ❑ |
| | | ▪ 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). | ❑ ❑ ❑ |

**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).