



Cryptosporidium: **An Auditor's Perspective**

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Why is Testing and Certification Important?

» **Public Safety**

» **Personal Safety**

» **It's Our JOB to Protect Public Health**



- » **On-line 4 Week Course**
- » **Comprehensive Final Exam**
- » **Mock Audit**
- » **Prerequisites**

The CO Course





LA OPH LAB





Fisherbrand
GLASS
CAUTION
Do not touch! Broken glassware
is sharp! Dispose of contents
and box at the same time for
maximum safety.





- » **On-site Audit – NJDEP May 2015**
- » **Drafting SOP's**
- » **Proficiency Testing**

Louisiana's Crypto Lab



QUALIFIED PERSONNEL

Position	Education	Experience with CRYPTO and FA Microscopy	Experience Using Method 1623/1623.1	Number of Samples Analyzed
Principal Analyst	BS/BA in Microbiology or closely related field	1 Year continuous	6 Months	100
Analyst	2 Years College in Microbiology or Equivalent	6 Months Continuous	3 Months	50
Technician	No Minimum Requirement	No Minimum Requirement	3 Months in Filter Extraction and Processing Protoza Samples	50

Analyst Requirements

» Monthly:

- > Count a slide prepared with 40-200 oocysts/cysts
- > Counts must be within 10%
- > Describe 10 oocysts/cysts using FITC, DAPI and DIC
 - + All differences discussed, resolved and documented

Analyst Verification



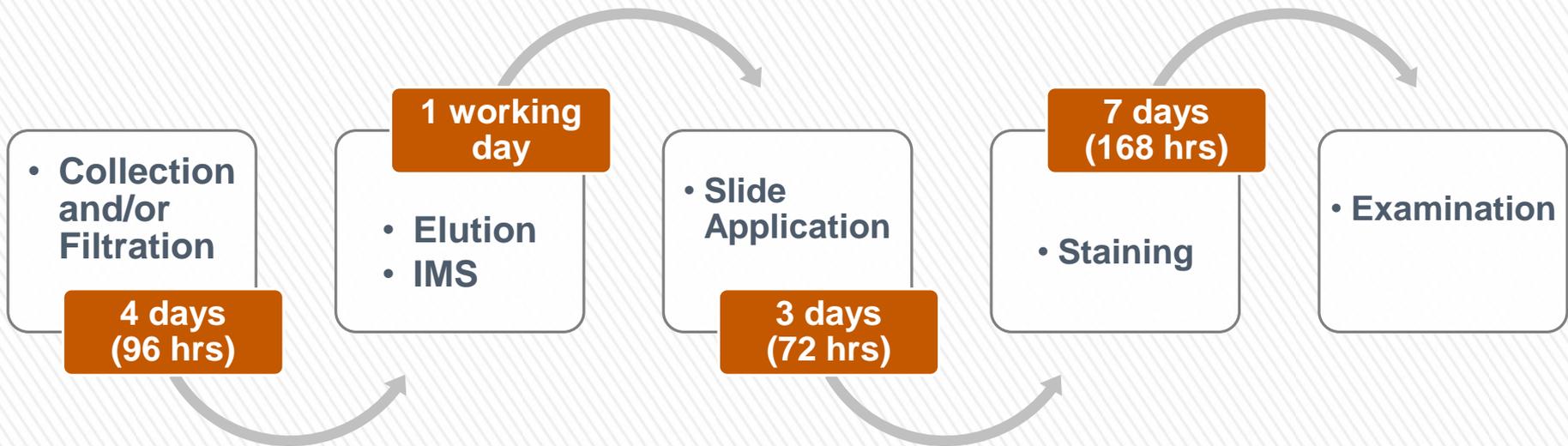
- » Key Component of Certification Process
- » Initial PT is a set of 8 Samples
- » 2 PT's Per Year (April and October)

Proficiency Testing Samples



Method Holding Times

Method 1623.1, Table 5



Method 1623/1623.1 Bench Sheet

Sample Identification Information

* Lab Sample ID: 1234-01	Turbidity (NTU): 2.9
* PWS ID: DH 123456	Person Receiving Sample: ST
* Facility ID: INX 723	Temperature (°C) @ sample receipt: 18
* Sample Collection Point ID: Intake 2	Date of sample receipt: 2-4-13
* Sample collection date & time: 2-4-13 0900	Time of Sample receipt: 0900
* Sample type (circle one): <input type="checkbox"/> Initial precision and recovery (IPR) <input type="checkbox"/> Method blank <input checked="" type="checkbox"/> Field (monitoring) sample <input type="checkbox"/> Ongoing precision and recovery (OPR) <input type="checkbox"/> Matrix spike (MS) <input type="checkbox"/> Proficiency testing (PT)	

Sample Spiking Information (for IPR, OPR, MS, and PT samples only)

* Estimated number spiked:	<small>Crypto</small>	-	<small>Giardia</small>	-	Spiking time: -
* Sample volume spiked (L):	-	-	-	-	Spiking date: -
Spike manufacturer & ID:	-	-	-	-	Spiking analyst: -

Sample Filtration

Filter type (circle one): Envirochek HV Filta-Max PCFC	Method version (circle one): 1623 1623.1
Did filter clog? (circle one): Yes No	Filtration time: 0915 Filter lot number: FW1234
* Number of filter(s) used?: 1	Filtration date: 2-4-13
* Volume filtered (L) to nearest 1/4L: 10	Filtration analyst: ST

Filter Elution (must be initiated within 96 hours of sample collection/filtration)

Elution procedure (circle one): Wrist shaker Filta-Max wash station	Elution Buffer lot number: 020413
Type of Elution buffer (circle one): NaHMP/LA-12 LA-12 PBST PCFC	
Elution buffer expiration date: 2-11-13	Elution time: 1200
NaHMP lot number: 011813	Elution date: 2-8-13
NaHMP expiration date: 8-18-13	Elution analyst: ST

Examination Date and Time

Method 1623/1623.1 Slide Examination Form

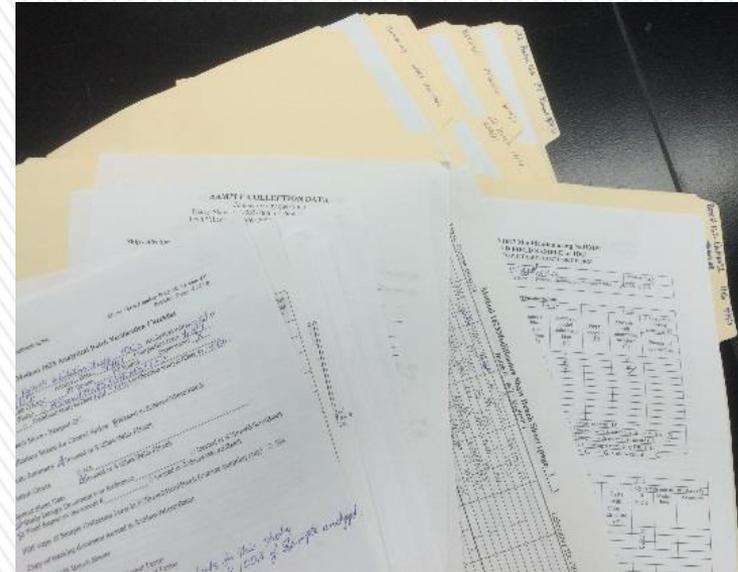
Sample ID: 1234-04	Analyst: Mico Queen
Examination/verification completion: (must be completed within 168 hours (7 days) of staining) Date: 2-12-13 Time: 0930	Slide number: 1 Total number of slides for this sample: 1
Positive staining control acceptable & 3 oocysts and cysts characterized with FITC, Size, Shape, DAPI and DIC <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	Negative staining control acceptable <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
FITC, Size, Shape, DIC and DAPI Characteristics Must Be Recorded for all Oocysts Detected in Field Sample <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	

Cryptosporidium Results

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim	Intense blue internal staining	Number of nuclei stained sky blue	Empty oocysts	Oocysts with amorphous structure	Oocysts with internal structure
								Number of sporozoites

Quality Control with Each Field Sample

- » OPR
- » Method Blank
- » Matrix Spike
- » Complete records for every positive result



Matrix Spike Sample Collection Site = Field Sample Collection Site

Frequency

- At least 1 matrix spike per 20 field samples

Critical OPR Elements

Analyzed before field samples

3 oocysts and 3 cysts characterized

Acceptance Criteria

- *Cryptosporidium* 33 – 100%
- *Giardia* 22 – 100%

Method Blank

Acceptance Criteria

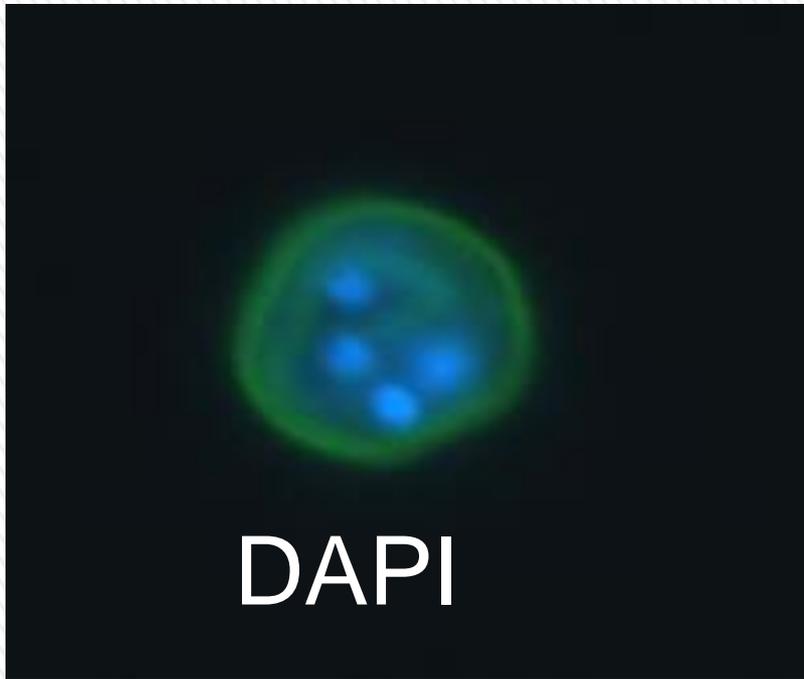
- Free of oocysts or cysts
- Free of potentially interfering organisms

Reagent Lots

Reagent Lot Numbers						
#	Spike	HV	EB	Na-HMP	IMS	Stain
OPR	B491	FW 1234	020413	011813	100100	B100
MB	-	“	“	“	“	“
FS 1	“	“	“	“	100101	“
FS 2	-	“	“	“	100101	“



Each positive result in a field sample must be fully characterized



Missed Opportunities

Cryptosporidium Results

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim	Intense blue internal staining	Number of nuclei stained sky blue	Empty oocysts	Oocysts with amorphous structure	Oocysts with internal structure Number of sporozoites
1	R	5.0 x 4.5	✓				✓	
2	R	4.5 x 4.5			3		✓	
3	R	5.0 x 5.0			2		✓	
4								
5								
6								
7								
8								
9								
10								
Total FA number from this slide: 12								
Analyst signature: <i>M. No. Jones</i>			P.A. <input checked="" type="checkbox"/>		Principal Analyst (P.A.) Signature: -			
Comments: -								

3 Key Items for Validation

1. Associated OPR $\geq 33\%$ recovery
2. Holding times must be met for field sample and QC
3. Each positive FA MUST be characterized



- » Enough Reagent for the Batch
- » Sooner Processed = Higher Quality of Data
- » Refrigerate at 1-10°C Not Frozen
- » Valid OPR Method Blank
- » Batches not Larger than 20 Samples
- » All Steps Properly Documented

Sample Management



- » All QC Records on Test Equipment and Reagents MUST be up to date
- » All Data Recorded in INK
- » Data Must be Retained for 5 Years
- » Electronic Files Must be Backed Up Regularly
- » PT Slides Retained for 60 Days

Data Management



- » Manage Time on Scope with Breaks
 - Microscope Fatigue is Real

- » Maintain Photo Library

- » Use Microscopy Training Modules

Microscope Time Management



- » Key QC Requirements are Noted as a **“MUST”**
- » Higher Skill Level Analysis for this Method than Conventional Microbiological Testing
- » Audits are Performed by the Observation of the Analysis

Summary





Questions

