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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

Office of Public Health Labor 1209

LA OPH LAB







» 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	BS/BA in	1 Year	6 Months	100
Analyst	Microbiology or closely related field	continuous		
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 1	623/1623.1 Bench Sheet
Sample	Identification Information
* Lab Sample ID: 1234-01	Turbidity (NTU): 2.9
* PWS ID: 04 123456	Person Receiving Sample: ST
* Facility ID: INX 7,23	Temperature (°C) @ sample receipt: /8
* Sample Collection Point ID. 19142	Date of sample receipt: 2-4/-13
* Sample collection date & time: 2-4-13 0900	Time of Sample receipt: 0900
* Initial precision and reco Sample type (circle one): Ongoing precision and re	ecovery (IPR) Method blank Field (monitoring) sample ecovery (OPR) Matrix spike (MS) Proficiency testing (PT)
Sample Spiking Information	on (for IPR, OPR, MS, and PT samples only)
* Estimated number spiked: Greater	Spiking time: -
* Sample volume spiked (L): -	Spiking date:
Spike manufacturer & ID:	Spiking analyst.
	Sample Filtration
Filter type (circle one): Envirochek HV Filta-Max PCI	FC Method version (circle one): 1623 (1623.1)
Did filter clog? (circle one): Yes No	Filtration time: 0915 Filter lot number: FW1231
* Number of filter(s) used?: /	Filtration date: 2-4-13
* Volume filtered (L) to nearest 1/4L: / ()	Filtration analyst: ST
Filter Elution (must be initiate	d within 96 hours of sample collection/filtration)
Elution procedure (circle one): (Wrist shaker Filta-Ma	x wash station Elution Buffer lot number: 0204/3
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: 01/8/3	Elution date: $2 - 8 - 13$
NaHMP expiration date: 3-18-13	Elution analyst:

Examination Date and Time

Method 1623/1623.1 Slide Examination Form

Sample ID: 1,234-04	Analyst Miceo Queen				
Examination/verification completion: (must be completed within 168 hours (7 days) of staining) Date: .2-1.2-1.3 Time: .0.130	Slide number:				
Positive staining control acceptable & 3 occysts and cysts characterized with FITC, Size, Shape, DAPI and DIC	Negative staining control acceptable				
FITC, Size, Shape, DIC and DAPI Characteristics Must Be Recorded for a	Il Oocynts Detected in Field Sample RYES INO				

Cryptosporidium Results

Object Shape located (oval by FA or No. round)	. ÷	hape oval L x W or ound)	DAPI -	D	API +		01	C .	
	Shape (oval		Light blue internal	intense blue	Intense blue Internal staining	Empty	Occysta with	Occysts with internal structure	
	or round)		staining, no distinct nuclei, green rim	internal staining		occysta	amorphous structure	Number of sporozoites	

Quality Control with Each Field Sample

- » <u>OPR</u>
- » Method Blank
- » Matrix Spike



» Complete records for every positive result

Quality Control Records

Bench Sheet

Laboratory Name:			Laboratory ID:					
Method 1623/1623 1 Bench Sheet								
Sample identification information								
* Lab Sample ID:	Lab Sample ID: Turbidity (NTU):							
PWS ID:			Person Receiving	a Sampler				
* Facility ID:			Temperature (*C)	@ sample receipt				
* Sample Collection Point ID:			Date of sample re	reiot				
Sample collection date & time:			Time of Sample re	eceipt				
•	nitial precision	n and recovery (IPR) Method bla	lank Field (monitoring) sample				
Sample type (circle one):	Ongoing preci	sion and recove	ry (OPR) Matrix	x spike (MS) Proficiency testing (PT)				
Sample	Spiking in	formation (f	OF IDR ODR	(vino seinmes TQ bne 2M				
* Estimated number spiked	* I	Oarda	Solving time	no, and P i campies enigr				
Sample volume spiked (L):	!		Spiking date:					
Spike manufacturer & ID:			Solking analyst					
The second se		san	pie Filtration					
Filter type (circle one): Envirochek i	HV Fita-N	Tax POPC	Method version (ci	Sircle one): 1623 1623.1				
Did filter clog? (circle one):	Yes	No	Fitration time:	Filter lot number.				
 Number of filter(s) used?. 			Filtration date:					
 Volume litered (L) to nearest 1/4L. 			Pitration analyst					
Filter Elution	n (must be	Initiated wi	thin 96 hours o	of sample collection/filtration)				
Elution procedure (circle one): W	vrist shaker	Filta-Max was	ih station	Elution Buffer lot number:				
Type of Elution buffer (circle one):		NaHMP/LA-12	2 LA-12	PBST PCFC				
Elution buffer expiration date:			Elution time:					
NaHMP lot number:			Elution date:					
NaHMP expiration date:			Elution analyst					
	Cond	centration, II	MS, and Silde I	Preparation				
(mus	t be compl	eted on same	e working day ti	that samples are eluted)				
Procedure (circle one):	Centrifuga	tion Fifta-Max	concentrator O	other (specify)				
 Pellet volume after concentration (mL) Total volume of peruppeded concentration 	.) to nearest t	1 mL:		Concentration analyst:				
 Total volume or resuspended concern Making of resuspended concerning 	trate (mL).	INC (ed.):		Olida presentise time:				
 Volume of resultablication concernance of humber of autoemplate processed the 	transferred to	net (mL).		Olide preparation units.				
IMS lot number	Number of subsamples processed through entire method.			Olde preparation caster.				
INC sector (circle one):	Dural OC	Combo Duni	ant Cauta Of	once preparation analysi.				
Sides (sinia ope) Meridian	Dynal GC-	Waterborne (arans-orypto or	(apecity)				
sides (croe one): Mendian Dynal Waterborne Other (specify)								
silde staining (mi	ust be con	npieted with	in 72 hours of	r application of sample to the slide)				
Detection kit (circle one): B	3TF EasyStai	n Merifluor	Crypt-a-glo Gi	iardi-a-glo Aqua-glo Other (specify)				
Detection kit lot number:	Detection kit lot number: Staining date & time:							
Number of slides for this sample:			Staring analyst:					
 Examination Results as Tota 	al FA num	ber from all	slides for sam	pie Cryptosporidium: Glardia:				
Comments:								

Slide Examination Form

Laboratory rorms.

Laboratory ID.

Method 1623/1623.1 Slide Examination Form

Sample D.	Analysi .				
Evanination/vennostion.completion	Sède number.				
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Positive alarang control acceptable 2.9 occysta and cysta chora che und with F.F.C., Sue, Stope, DAPI and D.C. D.Y.E.S. D.NO	Negalive sitering control acceptable D YE 3 D NO				
F IFC, Sue, Stage, DIC and DAPI Characteristics Musi Be Recorded for al	il Gocyala Delected in Field Sample D YES D NO				

DAPI-DAPL! D.I.C. Cibjed Iocaled Shape Joval Cocysia with internal Sum in la ra a Light blue internal Numbero Googala with siudu s LXW blue Emply by F.A. sisning to daind nucle sisred amorphous (pm0) op cycel at Ňa. 10000 nucle, green rum aky bian ahuchum Number of sportaneous alarıng . z 9 4 5 . r а 9 10 I del FAnunker von this slide Analysi segrature . P.A.O Punopal Analysi (P.A.)Signature . Comments.

	Giandia Results										
			DAPI-	DAPI- DAPI-		D.I.C.					
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Cryptosporidium Results

Laboratory Name:			Laboratory ID			
	M	ethod 1623	3/1623.1 Ber	nch Sheet		
		Sample Ide	ntification Info	ormation		
* Lab Sample ID: Turbidity (NTU):						
* PWS ID:			Person Receiving	3 Sample:		
* Facility ID:			Temperature (°C)	@ sample receipt:		
* Sample Collection Point ID:		a .	Date of sample re	eceipt:		
* Sample collection date & time:			Time of Sample r	receipt:		
* Initial precision and recovery (IPR) Method blank Field (monitoring) sample Ongoing precision and recovery (OPR) Matrix spike (MS) Proficiency testing (PT)						
Sam	ple Spiking I	nformation (for IPR, OPR,	MS, and PT samples only)		
* Estimated number spiked:	Crypto	Giardia	Spiking time:			
* Sample volume spiked (L):			Spiking date:			
Spike manufacturer & ID:			Spiking analyst:			
		Sar	nple Filtration			
Filter type (circle one): Enviroc	hek HV Filta-'	Max PCFC	Method version (circle one): 1623 1623.1		
Did filter clog? (circle one):	Yes	No	Filtration time:	Filter lot number:		
* Number of filter(s) used?:			Filtration date:			
* Volume filtered (L) to nearest 1/4	iL:		Filtration analyst:			
Filter Elu	tion (must b	e initiated wi	ithin 96 hours	of sample collection/filtration)		
Elution procedure (circle one):	Wrist shaker	Filta-Max wa	sh station	Elution Buffer lot number:		
Type of Flution buffer (circle one	ŀ	NaHMP/LA-1	12 I A-12	PBST PCEC		
Elution buffer expiration date:	<i>,.</i>		Elution time:			
NaHMP lot number:			Elution date:			
NaHMP expiration date:	-		Elution analyst:			
	Cor	contration	MS and Slido	Propagation		
(1	must be comr	pleted on sam	ne working day	that samples are eluted)		
Procedure (circle one):	Centrifug	ation Filta-Ma	x concentrator	Other (specify)		
* Pellet volume after concentration	(mL) to nearest	0.1mL:	Concentration analyst:			
* Pellet volume after concentration (mL) to nearest 0.1mL:				IMS analyst:		
* Total volume of resuspended cor	ncentrate (mL);			IMS analyst:		
* Total volume of resuspended cor * Volume of resuspended concent	ncentrate (mL): rate transferred t	o IMS (mL):		IMS analyst: Slide preparation time:		
 * Total volume of resuspended cor * Volume of resuspended concentre Number of subsamples processed 	ncentrate (mL): rate transferred t d through entire	o IMS (mL): method:		IMS analyst: Slide preparation time: Slide preparation date:		
* Total volume of resuspended cor * Volume of resuspended concent Number of subsamples processe IMS lot number:	ncentrate (mL): rate transferred t ad through entire	o IMS (mL): method:		IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst:		
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* Total volume of resuspended cor * Volume of resuspended concent Number of subsamples processe IMS lot number: IMS system (circle one): Slides (circle one): Mer	ncentrate (mL); rate transferred t ad through entire Dynal GC idian Dynal	o IMS (mL): method: C-Combo Dyn Waterborne	al anti-Crypto C Other (specify)	IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst: Sther (specify)		
Total volume of resuspended cor Volume of resuspended concent Number of subsamples processe IMS lot number: IMS system (circle one): Slides (circle one): Mer Slide Staining	rate transferred t ad through entire Dynal GC idian Dynal (must be co	o IMS (mL): method: C-Combo Dyn Waterborne	al anti-Crypto C Other (specify) hin 72 hours o	IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst: Other (specify)		
 Total volume of resuspended coronal Volume of resuspended concent Number of subsamples processe IMS lot number; IMS system (circle one); Slides (circle one); Mer Slide Staining Detection kit (circle one); 	ncentrate (mL): rate transferred t ed through entire Dynal GC idian Dynal (must be co BTF EasySta	o IMS (mL): method: C-Combo Dyn Waterborne mpleted wittl	al anti-Crypto C Other (specify) hin 72 hours o Crypt-a-olo G	IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst: Sther (specify) f application of sample to the slide) Siardi-a-glo Aqua-glo Other (specify)		
* Total volume of resuspended cor * Volume of resuspended concent Number of subsamples processe IMS lot number: IMS system (circle one): Slides (circle one): Slide Staining Detection kit (circle one): Detection kit (d number:	ncentrate (mL): rate transferred t ed through entire Dynal GC idian Dynal (must be co BTF EasySta	o IMS (mL): method: C-Combo Dyn Waterborne mpleted witi ain Merifluor	al anti-Crypto C Other (specify) hin 72 hours o Crypt-a-glo G Staining date & ti	IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst: Sther (specify) f application of sample to the slide) siardi-a-glo Aqua-glo Other (specify) me:		
Total volume of resuspended cord Volume of resuspended concent Number of subsamples processe IMS lot number: IMS system (circle one): Slides (circle one): Mer Slide Staining Detection kit (circle one): Detection kit of number: Number of slides for this sample	ncentrate (mL): rate transferred t d through entire Dynal GC idian Dynal (must be co BTF EasySta	o IMS (mL): method: C-Combo Dyn Waterborne mpleted witti ain Merifluor	al anti-Crypto C Other (specify) hin 72 hours o Crypt-a-glo C Staining date & ti Staining analyst	IMS analyst: Slide preparation time: Slide preparation date: Slide preparation analyst: Sther (specify) f application of sample to the slide) siardi-a-glo Aqua-glo Other (specify) me:		

QC Batch

* = Data entered into LT2/Stage2 Data Collection and Tracking System

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	-	66	66	"	"	"				
FS 1	"	"	"	"	100101	"				
FS 2	-	66	66	"	100101	"				

Each positive result in a field sample must be fully characterized





Missed Opportunities

Cryptosporidium Results

			DAPI -	DAPI +		D.I.C.		
Object located by FA	Shape (oval or	Size L x W	Light blue internal staining, no distinct	intense blue	Number of nuclei stained	Empty	Occysts with amorphous	Occyats with internal structure
No.	round)	(proj	nuclei, green rim	staining	sky blue	occysts	structure	Number of sporozoites
1	R	5.4+45	~				V	
2	R	1.5+4.5			.3		V	
3	R	Sex S.O			3		V	
4								
5								
6								
7								
8								
0								
18								
Total FA number from this slide: 12								
Analyst si	gnature: 🦷	Millio	Queen	PAE	Principal Analys	it (P.A.)Sign	iature: 💶	
Comment	s: —							

3 Key Items for Validation

- **1.** Associated OPR ≥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 Filed 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





Questions