NEW YORK STATE

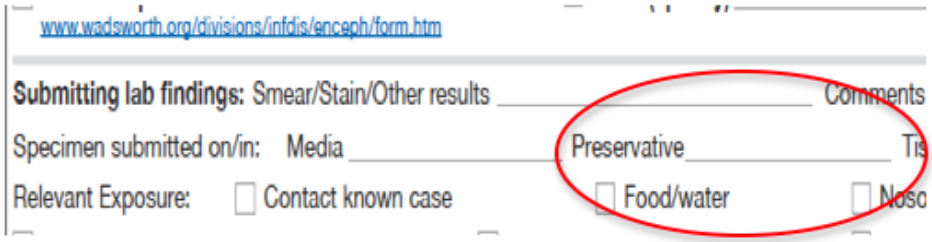
Parasitology Proficiency Testing Program

News and Notes

Beginning with the May 2013 event a separate set of 3 samples were supplied for laboratories performing antigen detection. For the November event these were samples 13I-K, 13I-L, and 13I-M. These are distinct from samples 13-K, 13-L, and 13-M and cannot be used interchangeably.

As molecular methods become increasingly common in the clinical parasitology lab, so does the necessity of knowing what preservative was used with the specimen. Preservatives commonly used for parasitology are not ideal for DNA extraction, and newly developed tests may only be approved for use with specific preservatives. For example, the assay may be approved for use with specimens preserved in 10% formalin but not SAF. Please remember to include the preservative information by filling out that section of the Infectious Disease Requisition, whenever submitting specimens to Wadsworth.

Please note that an unpreserved specimen should also be submitted, whenever possible, to maximize the likelihood of extracting good quality DNA.



Parasitology Comprehensive
5 November 2013

The purpose of the New York State Proficiency Testing Program in the category of Parasitology - Comprehensive is to monitor the performance of applicant laboratories that detect and identify parasites in fecal emulsions, fecal smears, and blood films. This document reports the results for the November 2013 proficiency test in Parasitology - Comprehensive and Antigen Detection.

Sample Preparation and Quality Control

All emulsions and slides used in this test were prepared by a commercial source. The emulsions were dispensed into the vials from pools, which were continuously mixed during the loading process. Numerous samples of each test specimen were selected at random by the Wadsworth Center Parasitology Laboratory of the New York State Department of Health, and were assayed for quality and confirmation of organisms. The supplying vendor also conducted extensive quality control tests and a detailed quality control report was submitted for inspection and verification. Samples were authenticated by at least 80% of participating laboratories and/or referee laboratories.

13-K (All Parasites)

Correct identification: *Cryptosporidium* sp.

Results of Participating Laboratories

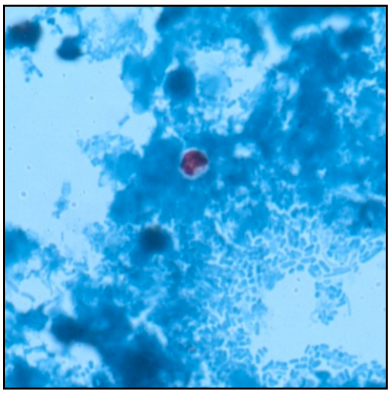
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Cryptosporidium sp.	85/100	85	10/10	**Correct**
Blastocystis hominis	5	5	0	**Incorrect**
No Parasites Seen	12	12	0	**Incorrect**

Quality Control and Referee Information

Participating and referee laboratories reported that ***Cryptosporidium* sp.** was the correct response (85 and 100% respectively). Quality control examination of 4% of the vials for this sample showed cysts in every 40X field. Other tests performed included a Direct Immunofluorescent Assay, which was positive for *Cryptosporidium* sp. and negative for *Giardia lamblia*. A modified acid-fast stained slide was also positive for *Cryptosporidium* species.

Please Note: As described in the instructions available on the website, you should include a test for *Cryptosporidium* sp. whenever required by the type of parasite requested. Thus, when "report all parasites" is indicated for a fecal emulsion, a test for *Cryptosporidium* should be performed.

Diagnostic Characteristics



***Cryptosporidium* sp.** has become one of the most important opportunistic infections seen in the immunocompromised patient. This coccidian parasite is spread through contaminated food or water. The diagnostic stage is the oocyst passed in the stool. On a modified acid-fast stained smear these oocyst measure 4-5 µm and stain pale to deep pink. It is sometimes possible to see the 4 sporozoites within the oocyst. Other methods of diagnosis include immunoassays and immunofluorescent stains.

13-L (Helminths Only)

Correct identification: *Diphyllobothrium latum*

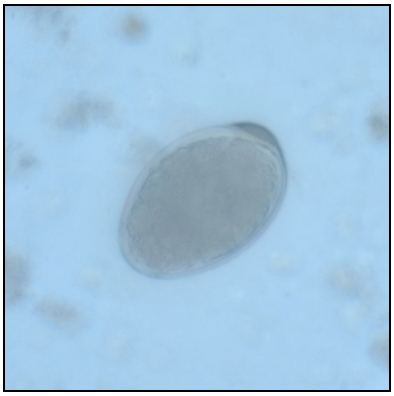
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Diphyllobothrium latum	99/100	99	10/10	**Correct**
Dientamoeba fragilis	1	1	0	**Incorrect**
No Parasites Seen	1	1	0	**Incorrect**

Quality Control and Referee Information

Participating and referee laboratories agreed that ***Diphyllobothrium latum*** was the correct response (99 and 100% respectively). Quality control examination of 4% of the vials for this sample showed an average of 30 ova per coverslip.

Diagnostic Characteristics



Diphyllobothrium latum is an intestinal tapeworm acquired by ingesting raw or poorly cooked freshwater fish. The diagnostic stage is the egg recovered in stool. These eggs are ovoid and measure 60-70 µm by 20-35 µm. They have an operculum at one end and a small knob at the other. The knob may or may not be visible depending upon the position of the egg. These eggs may be confused with *Paragonimus* sp. so measurement with a calibrated ocular micrometer is important.

13-M (Helminths Only)

Correct identification: *Clonorchis sinensis*

Results of Participating Laboratories

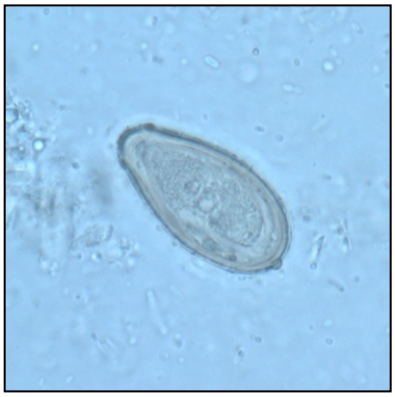
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Clonorchis sinensis / Opisthorchis sp. *	78/100	78	6/10	**Unauthenticated**
Taenia sp.	2	2	0	**No Penalty**
No Parasites Seen	20	20	4	**No Penalty**

* Sample contained *Clonorchis sinensis / Opisthorchis* sp. but was not authenticated.

Quality Control and Referee Information

Although the majority of participating and referee laboratories agreed that ***Clonorchis sinensis*** was the correct response (78 and 60% respectively), this sample was not authenticated by 80% of the participating or referee laboratories. Therefore this sample was graded as No Penalty. Quality control examination of 4% of the vials for this sample showed 1-2 ova per coverslip.

Diagnostic Characteristics



Clonorchis sinensis is a trematode that parasitizes the biliary ducts of humans. Humans become infected when they eat uncooked freshwater fish that contain metacercariae. The metacercariae excyst and travel to the distal bile capillaries where the worms mature. Adult worms deposit eggs in the bile fluid and these are later discharged into the feces. The eggs measure 28-35 µm. They are thick shelled, ovoid, have an operculum with distinct opercular shoulders, and a knob at the abopercular end.

13-N (All Parasites)

Correct identification: *Dientamoeba fragilis*

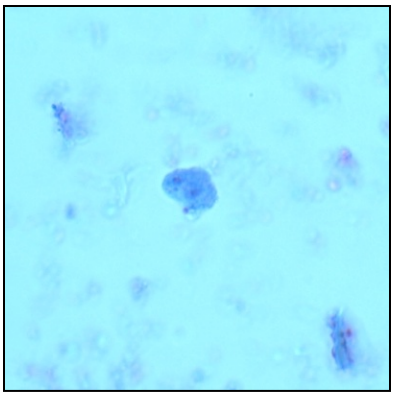
Results of Participating Laboratories

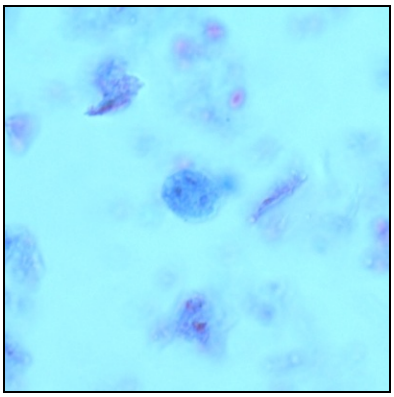
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Dientamoeba fragilis	91/100	91	8/10	**Correct**
Endolimax nana	6	6	2	**Incorrect**
Blastocystis hominis	3	3	0	**Incorrect**
Iodamoeba butschlii	2	2	0	**Incorrect**
No Parasites Seen	3	3	1	**Incorrect**

Quality Control and Referee Information

Participating and referee laboratories agreed that ***Dientamoeba fragilis*** was the correct response (91 and 80% respectively). Quality control examination of 4% of the slides for this sample showed an average of 1 trophozoite per 100X oil immersion field.

Diagnostic Characteristics





Dientamoeba fragilis is distributed worldwide. Despite the name, it is classified as a flagellate rather than an amoeba. There is no known cyst stage. Trophozoites are either uni- or bi-nucleated. Uni-nucleate organisms are easily confused with *Endolimax nana*. The nuclear chromatin can be fragmented, which is helpful in distinguishing this organism from *E. nana*, and no peripheral chromatin is seen. The cells themselves are amoeboid in shape and measure between 5-15 µm, with a typical range of 10-12 µm. The cytoplasm is finely granular and may contain vacuoles. The cell boundary may be difficult to discern on a trichrome stained specimen and the staining characteristics of this organism are quite variable.

13-O (All Parasites)

Correct identification: *Trypanosoma cruzi*

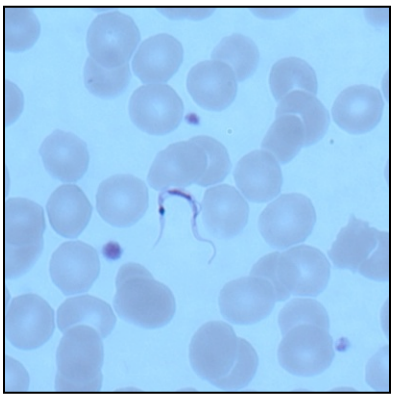
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Trypanosoma cruzi	96/100	96	10/10	**Correct**
Trypanosoma brucei	1	1	0	**Incorrect**
Test Not Performed	3	3	0	**No Penalty**

Quality Control and Referee Information

Participating and referee laboratories agreed that ***Trypanosoma cruzi*** was the correct response (96 and 100% respectively). Quality control examination of 4% of the slides for this sample showed an average of 3 organisms per 100X oil immersion field. Organisms have a central nucleus and a prominent kinetoplast.

Diagnostic Characteristics



Trypanosoma cruzi is the causative agent of the zoonosis Chagas' disease. It is a major health problem in Latin America. The organism is transmitted through the feces of the reduviid bug when it takes a blood meal. Trypomastigotes are detected in the blood on thin and thick smears. They measure approximately 20 µm long and usually are C or U shaped. The nucleus is located in the middle of the organism and a large kinetoplast (mitochondrial DNA) is located at the posterior end. A flagellum arises from the flagellar pocket near the kinetoplast and follows the undulating membrane to the anterior end where it projects as a free flagellum. On Giemsa-stained smears the cytoplasm stains bluish while the nucleus and kinetoplast stain purple or red. *Trypanosoma cruzi* is distinguished from *Trypanosoma brucei* primarily by the prominence of the kinetoplast, which is much larger in *Trypanosoma cruzi.*

A separate set of samples (13I-K, 13I-L, and 13I-M) was sent for antigen detection. These results are reported below.

Scoring Information

Immunoassay Results

Cryptosporidium	**13I-K**		**13I-L**		**13I-M**	
METHOD	**-**	**+**	**-**	**+**	**-**	**+**
MCC Para-Tect Cryptosporidium/Giardia DFA	0	1	1	0	0	1
Meridian ImmunoCard STAT Cryptosporidium/Giardia	0	25	25	0	0	25
Meridian Merifluor Cryptosporidium/Giardia	0	17	17	0	0	17
Meridian Premier Cryptosporidium	0	1	1	0	0	1
Remel ProSpecT Cryptosporidium EIA	0	14	14	0	1	13
Remel Xpect Giardia/Cryptosporidium	0	6	6	0	0	6
TechLab Cryptosporidium II ELISA	0	2	2	0	0	2
TechLab Giardia/Cryptosporidium Quik Chek (Alere)	0	8	8	0	0	8
TechLab/Wampole Test EIA	0	4	4	0	0	4

Giardia	**13I-K**		**13I-L**		**13I-M**	
METHOD	**-**	**+**	**-**	**+**	**-**	**+**
MCC Para-Tect Cryptosporidium/Giardia DFA	1	0	0	1	0	1
Meridian ImmunoCard STAT Cryptosporidium/Giardia	25	0	0	25	0	25
Meridian Merifluor Cryptosporidium/Giardia	13	0	1	12	0	13
Meridian Premier Giardia	1	0	0	1	0	1
Remel ProSpecT Giardia EIA	21	0	1	20	1	20
Remel ProSpecT Giardia EZ	2	0	0	2	0	2
Remel Xpect Giardia	2	0	0	2	0	2
Remel Xpect Giardia/Cryptosporidium	6	0	0	6	0	6
TechLab Giardia II ELISA	2	0	0	2	0	2
TechLab Giardia/Cryptosporidium Quik Chek (Alere)	8	0	0	8	0	8
TechLab/Wampole Test EIA	7	0	0	7	0	7

Distribution of Scores

Score	# of labs	% of labs
100	72	71
90-99	3	3
80-89	18	18
70-79	5	5
60-69	2	2
0-59	1	1

Answer Key Parasitology - Comprehensive

Sample	Correct Answer
13-K	*Cryptosporidium* sp.
13-L	*Diphyllobothrium latum*
13-M	*Dientamoeba fragilis*
13-N*	*Clonorchis sinensis / Opisthorchis* sp.*
13-O	*Trypanosoma cruzi*

* Sample contained *Clonorchis sinensis / Opisthorchis* sp. but was not authenticated. Therefore this sample was graded as No Penalty.

Answer Key Parasitology - Antigen Detection

Sample	Correct Answer
13I-K	*Cryptosporidium* sp.
13I-L	*Giardia lamblia*
13I-M	*Cryptosporidium* sp. and *Giardia lamblia*

Grading

The answer key was derived from the response of all participating laboratories as per **CLIA Regulations**, CFR Title 42, Part 493, Subpart I, Section 493.917. These regulations can be viewed at www.cdc.gov/clia/Regulatory/default.aspx. These regulations state that 80% or more of participating laboratories **or** referee laboratories must identify the parasite for it to be authenticated as a correct answer. Similarly, reporting of a parasite identified by less than 10% of the participating laboratories **or** referees is an incorrect response. Organisms that are not authenticated, but which were reported by more than 10% of the participating laboratories **or** referees, are "Unauthenticated" and are not considered for grading.

Credit is given according to the formula:

[# of Correct Responses / (# of Correct Responses + # of Incorrect Responses)] X 100

For example, if a sample contained one principal parasite and the laboratory reported it correctly but reported the presence of an additional parasite, which was not present, the sample grade would be:

$1/(1+1)\times 100 = 50$ percent.

Important Reminders

The next Parasitology Proficiency Test is scheduled for **February 4, 2014.** You are responsible for notifying us **before February 11, 2014** if you do not receive your samples. Proficiency test results must be electronically submitted through EPTRS by **February 19, 2014** or the laboratory will receive a score of zero. These requirements are stated in the NYS Proficiency Testing Program Guide provided by the NYS Clinical Laboratory Evaluation Program, which can be accessed via the Internet at:

http://www.wadsworth.org/labcert/clep/ProgramGuide/pg.htm