

NEW YORK STATE

Parasitology Proficiency Testing Program

News and Notes

Samples supplied for laboratories performing antigen detection are 15I-F, 15I-G, and 15I-H. These are distinct from samples 15-F, 15-G, and 15-H 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: Specimens preserved in ethanol based fixatives (e.g. Total-Fix or Eco-Fix) or an unpreserved specimen should also be submitted, whenever possible, to maximize the likelihood of extracting good quality DNA.

The image shows a screenshot of a web-based form from Wadsworth Center. The URL at the top is www.wadsworth.org/divisions/infdis/enceph/form.htm. The form contains several fields: 'Submitting lab findings: Smear/Stain/Other results' with a 'Comments' field to its right; 'Specimen submitted on/in: Media' and 'Preservative' fields; and a 'Relevant Exposure:' section with checkboxes for 'Contact known case', 'Food/water', and 'Nose'. The 'Preservative' field is circled in red.

Parasitology Comprehensive 19 May 2015

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. Below please find the results for the May 2015 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 (NYSDOH), 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.

15-F (Helminths Only)

Correct Identification: No Parasites Seen

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
No Parasites Seen	90/90	100	10/10	Correct

Quality Control and Referee Information

All participating and referee laboratories agreed that **No Parasites Seen** was the correct response. Quality control examination of 4% of the vials for this sample showed normal fecal elements and no organisms present. Other tests performed included a direct immunofluorescent assay for *Giardia lamblia* and *Cryptosporidium* sp., which was negative for both organisms and a modified acid-fast stained slide, which was also negative.

15-G (Helminths Only)

Correct Identification: *Strongyloides stercoralis*

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
<i>Strongyloides stercoralis</i>	88/90	98	10/10	Correct
<i>Necator americanus</i> / <i>Ancylostoma duodenale</i> (Hookworm)	2	2	0	Incorrect

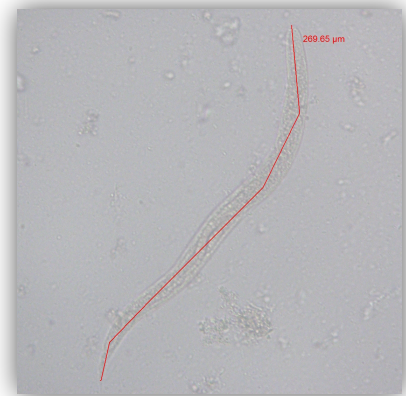
Quality Control and Referee Information

Participating and referee laboratories agreed that *Strongyloides stercoralis* was the correct response (98 and 100% respectively). Quality control examination of 4% of the vials for this sample showed an average of 4 organisms per coverslip.

Diagnostic Characteristics

The diagnostic stage of *Strongyloides stercoralis* is the rhabditiform larvae passed in the stool. Larvae measure 180-380 μm , have a short buccal cavity, and a prominent genital primordium (indicated by the arrow in the image below).

Strongyloides stercoralis is an intestinal nematode with a very complex life cycle. Infection is acquired when filariform larvae in the soil penetrate the skin and are carried through the blood to the lungs. From the lungs they travel up the trachea and are swallowed. Once in the intestine they develop into mature female worms and begin to produce eggs by parthenogenesis. These eggs, which are rarely seen, hatch in the intestine into rhabditiform larvae. The larvae pass in the feces and develop into male and female worms in the soil where they complete their life cycle.



15-H (All Parasites)

Correct Identification: *Balantidium coli*

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
<i>Balantidium coli</i>	89/90	99	10/10	Correct
No Parasites Seen	1	1	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that *Balantidium coli* was the correct response (99 and 100% respectively). Quality control examination of 4% of the vials for this sample showed an average of 1 organism per 100X field.

Diagnostic Characteristics

Balantidium coli is the only pathogenic ciliate to infect humans and the largest of all protozoa. The trophozoites measure 50-100 μm by 20-50 μm and are completely covered with cilia. They have a large bean-shaped macronucleus and a second smaller, round micronucleus. The characteristic bean shaped macronucleus can be seen in the image on the right below. The anterior end of the organism is slightly pointed and in many cases the cytosome is visible. No cysts were seen in quality control examination of this specimen. When present cysts measure 50-70 μm and also have a macro- and micronucleus.

Pigs are a natural reservoir for *B. coli* and contact with pigs is a risk factor for infection with this parasite.



15-I (All Parasites)

Correct Identification: *Entamoeba hartmanni*

Results of Participating Laboratories

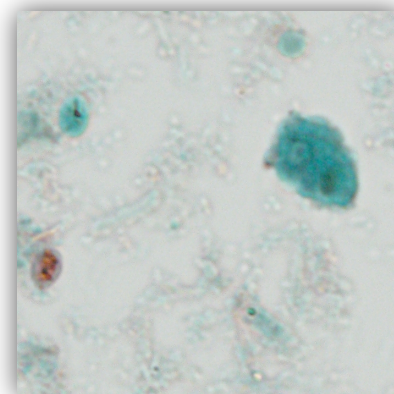
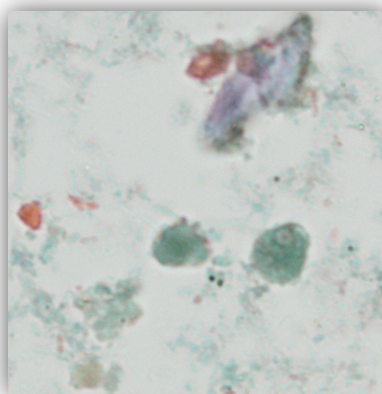
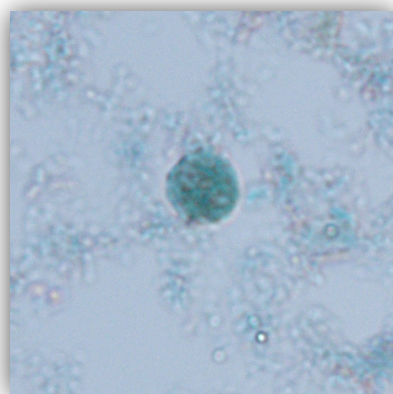
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
<i>Entamoeba hartmanni</i>	84/90	93	10/10	Correct
<i>Entamoeba histolytica/dispar</i>	6	7	0	Incorrect
<i>Entamoeba coli</i>	1	1	0	Incorrect
<i>Dientamoeba fragilis</i>	1	1	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that *Entamoeba hartmanni* was the correct response (93% and 100% respectively). Quality control examination of 4% of the slides for this sample showed an average of 5 organisms in every 40X field.

Diagnostic Characteristics

This sample contained trophozoites of *Entamoeba hartmanni*. This intestinal amoeba is morphologically similar to *E. histolytica*. The primary difference is a smaller size, usually $<10\ \mu\text{m}$ for *E. hartmanni* vs. $10\text{--}15\ \mu\text{m}$ for *E. histolytica/dispar* on a stained slide. Cysts are small, measuring $5\text{--}8\ \mu\text{m}$ and contain from 1 to 4 nuclei with centrally located karyosomal chromatin and fine, evenly distributed peripheral chromatin. Trophozoites measure from $5\text{--}10\ \mu\text{m}$ and contain 1 nucleus. *Entamoeba hartmanni* is considered a nonpathogenic parasite.



15-J (All Parasites)

Correct Identification: *Plasmodium falciparum*

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
<i>Plasmodium falciparum</i>	72/85	85	10/10	Correct
<i>Plasmodium malariae</i>	5	6	0	Incorrect
<i>Plasmodium ovale</i>	2	2	0	Incorrect
<i>Plasmodium vivax</i>	1	1	0	Incorrect
<i>Plasmodium vivax/ovale</i>	1	1	0	Incorrect
<i>Plasmodium</i> species *	3	4	0	Correct *
Parasites Seen *	1	1	0	Correct *

* Would Refer Per SOP (Only for laboratories that do not identify to species).

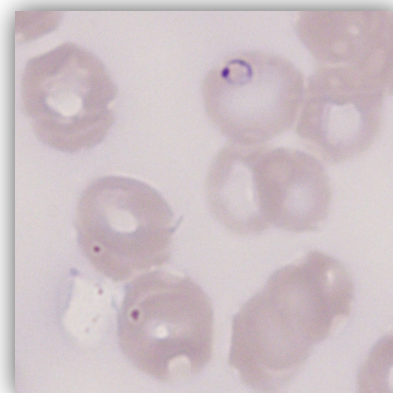
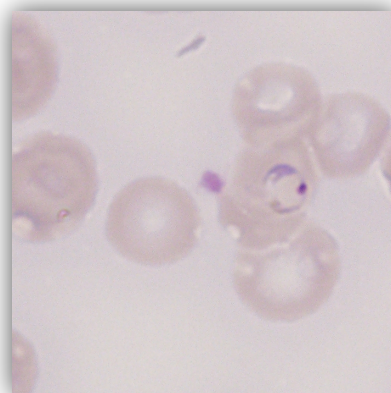
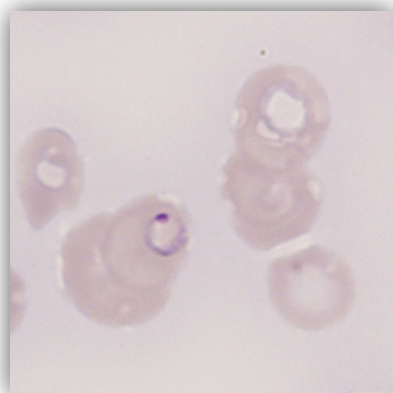
Quality Control and Referee Information

Participating and referee laboratories agreed that *Plasmodium falciparum* was the correct response (85 and 100% respectively). Quality control examination of 4% of the slides for this sample showed numerous trophozoites, an average of 1-2 organisms per 100X oil immersion field. Staining quality was good.

Diagnostic Characteristics

Plasmodium falciparum causes the most severe malaria as the parasite invades all ages of red blood cells and has a reproductive cycle of 36-48 hours. Parasitemia can reach up to 30%. For *P. falciparum*, the stage seen in peripheral blood is early trophozoites, or rings and gametocytes. As is common for *P. falciparum*, both cells with more than one ring and applique forms were present in this specimen. The presence of only early trophozoites and a high parasitemia are also characteristic of this species.

While *P. falciparum* and *Babesia* can have similar features several key differences distinguish them. *B. microti* rings are typically smaller and cells may contain 3 or 4 parasites. Applique forms are common to *P. falciparum* but not *Babesia*. Extracellular parasites are only seen with *Babesia* and pigment is only observed with *Plasmodium*.



Parasitology Antigen Detection

A separate set of samples (15I-F, 15I-G, and 15I-H) was sent for antigen detection. These results are reported below and show that the labs testing for *Cryptosporidium* and/or *Giardia* (99%, 100% respectively) obtained the correct answers for all three specimens.

Immunoassay Results

<i>Cryptosporidium</i>	15I-F		15I-G		15I-H	
METHOD	-	+	-	+	-	+
Cardinal Health Crypto Giardia Test Kit	0	1	0	1	1	0
MCC Para-Tect Cryptosporidium/Giardia DFA	0	2	0	2	2	0
Meridian ImmunoCard STAT Cryptosporidium/Giardia	0	17	0	17	17	0
Meridian Merifluor Cryptosporidium/Giardia	0	15	0	15	14	1
Meridian Premier Cryptosporidium	0	1	0	1	1	0
Remel ProSpecT Cryptosporidium EIA	0	10	0	10	10	0
Remel Xpect Giardia/Cryptosporidium	0	5	0	5	5	0
TechLab Cryptosporidium II ELISA	0	1	0	1	1	0
TechLab Giardia/Cryptosporidium Quik Chek (Alere)	0	12	0	12	12	0
TechLab/Wampole Test EIA	0	4	1	3	4	0

# of labs reporting	0/0	68/68	1/68	67/68	67/68	1/68
% of labs reporting	0	100	1	99	99	1
Status	Correct	Correct	Incorrect	Correct	Correct	Incorrect

<i>Giardia</i>	15I-F		15I-G		15I-H	
METHOD	-	+	-	+	-	+
Cardinal Health Crypto Giardia Test Kit	0	1	1	0	1	0
MCC Para-Tect Cryptosporidium/Giardia DFA	0	2	2	0	2	0
Meridian ImmunoCard STAT Cryptosporidium/Giardia	0	17	17	0	17	0
Meridian Merifluor Cryptosporidium/Giardia	0	12	12	0	12	0
Meridian Premier Giardia	0	1	1	0	1	0
Remel ProSpecT Giardia EIA	0	18	18	0	18	0
Remel Xpect Giardia	0	1	1	0	1	0
Remel Xpect Giardia/Cryptosporidium	0	5	5	0	5	0
TechLab Giardia II ELISA	0	1	1	0	1	0
TechLab Giardia/Cryptosporidium Quik Chek (Alere)	0	12	12	0	12	0
TechLab/Wampole Test EIA	0	6	6	0	6	0

# of labs reporting	0/0	76/76	76/76	0/0	76/76	0/0
% of labs reporting	0	100	100	0	100	0
Status	Correct	Correct	Correct	Correct	Correct	Correct

Scoring Information

Distribution of Scores Parasitology - Comprehensive

Score	# of labs	% of labs
100	74	82
90-99	0	0
80-89	12	13
70-79	1	1
60-69	2	2
40-59	0	0
0-39	1	1

Distribution of Scores Parasitology - Antigen Detection

Score	# of labs	% of labs
100	74	97
90-99	0	0
80-89	2	3
70-79	0	0
60-69	0	0
0-59	0	0

Answer Key Parasitology - Comprehensive

Sample	Correct Answer
15-F	No Parasites Seen
15-G	<i>Strongyloides stercoralis</i>
15-H	<i>Balantidium coli</i>
15-I	<i>Entamoeba hartmanni</i>
15-J	<i>Plasmodium falciparum</i>

Answer Key Parasitology - Antigen Detection

Sample	Correct Answer
15I-F	<i>Cryptosporidium</i> sp. and <i>Giardia lamblia</i>
15I-G	<i>Cryptosporidium</i> sp.
15I-H	Negative

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:

$$[\# \text{ of Correct Responses} / (\# \text{ of Correct Responses} + \# \text{ of Incorrect Responses})] \times 100$$

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:

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

Important Reminders

The next Parasitology Proficiency Test is scheduled for **October 6, 2015**. Participating labs will need to notify us **before October 13, 2015** if the samples are not received. Proficiency test results must be electronically submitted through EPTRS by **October 21, 2015** or the laboratory will receive a score of zero. This and additional information can be found 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>