NEW YORK STATE *Parasitology Proficiency Testing Program*

Parasitology General 17 May 2011

The purpose of the New York State Proficiency Testing Program in the category of Parasitology (General) is to monitor the performance of applicant laboratories in detecting and identifying parasites in fecal emulsions, fecal smears, and blood films. This document reports the results for the May 2011 proficiency test in Parasitology General.

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 Parasitology Unit of the David Axelrod Institute for Public Health, Wadsworth Center, and were assayed for quality and confirmation of contents. Extensive quality control tests were also conducted by the supplying vendor and a detailed quality control report was submitted to the New York State Parasitology Laboratory for inspection and verification. Samples were authenticated by 80% of participating laboratories and/or referee laboratories.

11-F (Helminths Only)

Correct diagnosis: *Hymenolepis nana*.

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Hymenolepis nana	110/110	100	10/10	Correct

Quality Control and Referee Information

Participating and referee laboratories agreed that *Hymenolepis nana* was the correct response (100%). Quality control examination of 4% of this sample showed an average of 20 ova per coverslip. Other tests performed include a Direct Immunofluorescent Assay and ELISA for *Giardia lamblia* and *Cryptosporidium* sp. which were negative for both organisms. A modified acid-fast stained smear was also negative.

Diagnostic Characteristics

Hymenolepis nana, also known as the dwarf tapeworm, is an intestinal cestode acquired by ingesting eggs from the environment or, rarely, by ingesting infected beetles. *H. nana* is the only



human tapeworm that doesn't have an intermediate host: transmission occurs from person to person. It has a worldwide distribution and is more commonly seen in children.

The diagnostic stage is the egg recovered in stool. These eggs are spherical, thin shelled, and measure 30 to 47μ m in diameter. They have a six- hooked oncosphere (the embryonic tapeworm) with two polar thickenings; the filaments that arise from these thickenings attach the embryo to the outer shell. Eggs of *H. nana* morphologically resemble those

of *Hymenolepis diminuta*. The primary difference is size: *H. diminuta* eggs are about twice the size (70-85µm) of *H. nana* eggs. Careful measurement with a calibrated ocular micrometer is essential.

11-G (Helminths Only)

Correct diagnosis: Ascaris lumbricoides.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Ascaris lumbricoides	104/110	95	8/10	Correct
No Parasites Seen	6	5	2	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that *Ascaris lumbricoides* was the correct response (95 and 80%). Quality control examination of 4% of this sample showed an average of 8 ova per coverslip. Both fertile and infertile eggs were present. Other tests performed include a Direct Immunofluorescent Assay and ELISA for *Giardia lamblia* and *Cryptosporidium* sp. which were negative for both organisms. A modified acid-fast stained smear was also negative.

Diagnostic Characteristics



Ascaris lumbricoides is one of the most common intestinal nematode infections of man. It is most prevalent in warm moist climates but can also be found in cooler areas. Infection is acquired when embryonated eggs in contaminated soil are ingested. The fertilized eggs (left image) are round to oval, mammillated, and golden brown in color. They measure 45-75µm by 35-50µm. Occasionally they may lose their outer mammillated layer. Infertile eggs (right image) are larger, less broad, and have thinner shells. They measure 85-90µm by 43-47µm.

11-H (Helminths Only)

Correct diagnosis: Hookworm.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Necator americanus/ Ancylostoma duodenale	110/110	100	10/10	Correct

Quality Control and Referee Information

Participating and referee laboratories agreed that Hookworm was the correct response (100%). Quality control examination of 4% of this sample showed an average of 15 ova per coverslip. Other tests performed include Direct Immunofluorescent Assay which was negative for both *Giardia lamblia* and *Cryptosporidium* sp and ELISA for *Giardia lamblia* and *Cryptosporidium* sp. which was negative for *Giardia lamblia* and indeterminate for *Cryptosporidium* sp.. A modified acid-fast stained smear was also negative.

Diagnostic Characteristics



Hookworm infection occurs in warm moist areas through skin penetration of filariform larvae from the soil. The larvae migrate through the heart and lungs, are swallowed, and take up residence in the small intestine where the adults mature.

The diagnostic stage is the egg passed in stool. They are oval and measure approximately 60 X 40 μ m. They have a thin shell with a space between the shell and the developing embryo. Development is usually at the 8 to 32 cell stage. The eggs of the two major species of hookworm (*Necator americanus* and *Ancylostoma duodenale*) cannot

be distinguished microscopically.

11-I (Protozoa Only)

Correct diagnosis: No Parasites Seen.

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Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
No Parasites Seen	108/110	98	10/10	Correct
Chilomastix mesnili	1	1	0	Incorrect
Endolimax nana	1	1	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that **No Parasites Seen** was the correct response (98 and 100%). Quality control examination of 4% of this sample showed no parasitic organisms present. The sample contained a large number of yeast cells, which are in the same size range as some smaller intestinal protozoa.

11-J (All Parasites)

Correct diagnosis: Plasmodium ovale.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium ovale	53/105	50	5/10	Correct
No Parasites Seen	26	25	4	No Penalty
Plasmodium vivax	13	12	1	No Penalty
Plasmodium malariae	10	10	0	No Penalty
Plasmodium sp.	3	3	0	No Penalty

Quality Control and Referee Information

Participating and referee laboratories failed to agree that *Plasmodium ovale* was the correct response (50%). Quality control examination of 4% of this sample showed parasites in every 30-40 100X oil immersion fields. Infected cells were somewhat enlarged, fimbriate, and had Schüffner's stippling. The organisms had compact cytoplasm and prominent chromatin.

Diagnostic Characteristics



Plasmodium ovale infections occur primarily in Central West Africa and some South Pacific Islands and account for fewer than 5% of all malaria cases. *P. ovale* malaria is usually less severe than other malarias and often ends in spontaneous recovery. The infected cells are usually enlarged,

although they tend to be smaller and with a more

regular outline than cells infected with *P. vivax*. Infected erythrocytes are also usually fimbriate (= having a "spiky" periphery), and have Schüffner's stippling. The cytoplasm of the trophozoites is generally less amoeboid than then that of *P. vivax* and the schizonts have 4-12 merozoites compared to 12-24 for *P. vivax*. The chromatin is usually very pronounced and the pigment is coarse.

Scoring Information

Cryptosporidium	11-F		11-G		11-H	
METHOD	-	+	-	+	-	+
Meridian ImmunoCard STAT Cryptosporidium/Giardia	27	0	27	0	27	0
Meridian Merifluor Cryptosporidium/Giardia	15	2	17	0	17	0
Remel ProspecT Cryptosporidium EIA	19	1	20	0	18	2
TechLab Cryptosporidium II ELISA	1	0	1	0	1	0
Remel Xpect Giardia/Cryptosporidium	5	0	5	0	5	0
TechLab/Wampole Test EIA	4	1	5	0	5	0

Immunoassay Results

Giardia	11	-F	11	-G	11	-H
METHOD	-	+	-	+	-	+
Meridian ImmunoCard STAT Crypto/Giardia	27	0	27	0	27	0
Meridian Merifluor Crypto/Giardia	12	1	13	0	13	0
Remel ProspecT Giardia EIA	20	5	25	0	24	1
Remel ProSpect Giardia EZ	2	0	2	0	2	0
Remel Xpect Giardia	2	0	2	0	2	0
Remel Xpect Giardia/Cryptosporidium	5	0	5	0	5	0
TechLab/Wampole Test EIA	7	1	8	0	8	0
TechLab Giardia II ELISA	1	0	1	0	1	0

Distribution of Scores

Score	# of labs	% of labs
100	117	94
90-99	0	0
80-89	5	4
70-79	1	1
60-69	1	1

Answer Key

Sample	Correct Answer	Points
11-F	Hymenolepis nana	20
11-G	Ascaris lumbricoides.	20
11-Н	Hookworm	20
11-I	No Parasites Seen	20
11-J	Plasmodium ovale*	20
TOTAL POSSIBLE POINTS	100 *unauthent	ticated

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

Each sample has a maximum value of 20 points. Credit is given according to the formula:

(# of Correct Responses / (# of Correct Responses + # of Incorrect Answers)) X 100

Important Reminders

The next Parasitology Proficiency Test is scheduled for **October 4, 2011.** You are responsible for notifying us **before October 11, 2011** if you do not receive your samples. Proficiency test results must be electronically submitted through EPTRS by **October 18, 2011** or the laboratory will receive a score of zero. These requirements are stated in the NYS Proficiency Testing Handbook provided by the NYS Clinical Laboratory Evaluation Program or can be accessed via the Internet at: http://www.wadsworth.org/labcert/clep/ProgramGuide/pg.htm

News and Notes

Beginning with the February 2009 proficiency exam, the **grading policy changed**. In order to make the score on the NYS Parasitology PT exam more accurately reflect laboratory performance, and be more consistent across categories, a new scoring system was put into effect. Under the new scoring system, grades are based only on the specimen or organism types processed by your laboratory.

Recently, there have been reports of high false-positive rates for *Cryptosporidium* by laboratories that use rapid immunochromatographic assays (Robinson et al. 2010). Additionally, the CDC has changed their case definition such that test results known to be obtained with commercially available immunochromatographic card tests are limited to meeting "probable" case criteria due to a recent report of unacceptably high rates of false-positive results. The Wadsworth Center Parasitology Laboratory has also observed that in some cases, samples submitted as positive for *Cryptosporidium* by a rapid immunochromatographic test fail to confirm by other methods. We are currently engaged in a study to determine whether preservative type, temperature or other factors can explain these discrepancies. Results will be reported in future publications by the laboratory.

Robinson TJ, Cebelinski EA, Taylor C, and Smith KE (2010) Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis. Clinical Infectious Diseases 50:e53-e55.