NEW YORK STATE

Parasitology Proficiency Testing Program

Blood Borne Parasites
17 May 2011

The purpose of the New York State Proficiency Testing Program in the category of Blood Borne Parasites is to monitor the performance of applicant laboratories in detecting and identifying parasites on blood films. This document reports the results for the May 2011 proficiency test in Blood Borne Parasites.

Sample Preparation and Quality Control

All slides used in this test were prepared and stained by a commercial source. 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.

11B-F

Correct diagnosis: *Plasmodium ovale*.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium ovale	6/28	21	4/10	Correct
Plasmodium vivax	8	29	3	No Penalty
Plasmodium malariae	5	18	3	No Penalty
Plasmodium falciparum	1	4	0	No Penalty
Parasites Seen	6	21	0	Correct
No Parasites Seen	2	7	0	Incorrect

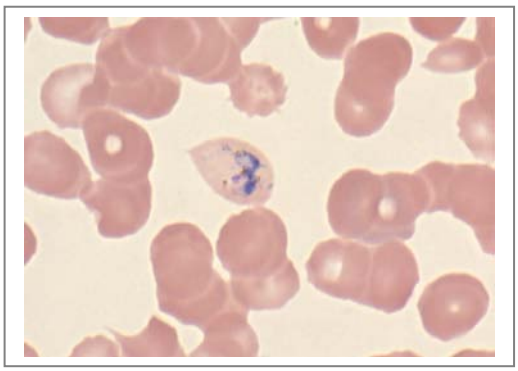
Quality Control and Referee Information

Participating and Referee laboratories agreed that parasites were present in this sample (92 and 100%) but failed to correctly identify it as ***Plasmodium ovale.*** For laboratories that perform parasite identification, the sample was authenticated to the genus level, so answers reporting any member of the genus *Plasmodium* were accepted. For those that perform parasite screens, the sample was authenticated, and therefore "Parasites Seen" was the correct answer.

Quality control examination of 4% of this sample showed parasites in every 10-12 100 X oil immersion fields. The infected cells are somewhat enlarged and the cytoplasm of the organisms is compact. The overall staining quality is good.

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.

11B-G

Correct diagnosis: *Loa loa.*

Results of Participating Laboratories

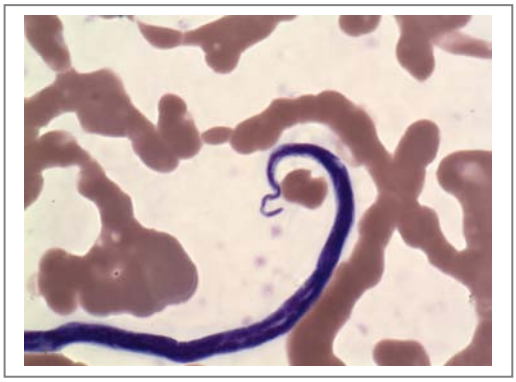
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Loa loa	9/28	32	5/10	Correct
Mansonella sp.	11	39	5	No Penalty
Microfilaria seen	1	4	0	Correct
Parasites Seen	1	4	0	Correct
No Parasites Seen	6	21	0	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that parasites were present in this sample (100%) but failed to correctly identify it as ***Loa loa*** (50%). Quality control examination of 4% of this sample showed an average of 9 organisms per slide. They measured approximately 6-7 µm wide by 200-250 µm long and had nuclei extending all the way to the tail tip. The diagnosis was complicated by the fact that the majority of the organisms had lost their sheath, although sheathed individuals were present. The staining quality is good.

Diagnostic Characteristics

Loa loa, also called the African eye worm, infects humans when they are bitten by infected deer or mango flies. The larvae are deposited into the bite wound and develop into adults within 6-12 months. Adults migrate beneath the conjunctiva or the eye, or through subcutaneous tissues. Years after the initial infection, the adults give rise to microfilariae which can be detected in the blood.



Microfilariae are sheathed and measure between 230-250 µm. They have nuclei that extend all the way to the tip of the tail, as shown in the image on the left. The sheath does not stain with Giemsa, and is usually visible as a clear zone surrounding the microfilarid. The sheath can be lost, making it more difficult to distinguish *Loa loa* from *Mansonella perstans.* However, *Mansonella perstans* is much smaller than *Loa loa* (see table below), which helps to prevent confusion.

Characteristics for Differentiating Microfilaria

	Brugia malayi	*Loa loa*	*Mansonella* sp.	*Wuchereria bancrofti*
Sheath	Present	Present	Absent	Present
Length	177-230 µm	230-250 µm	163-203 µm	244-296 µm
Width	5-6 µm	5-7 µm	3-5 µm	7-10 µm
Nuclei/Tail	Subterminal and terminal nuclei	Nuclei extend to the tip of the tail	Species dependent	No nuclei in tail
Key Features	Sheath stains pink with Giemsa, terminal and subterminal nuclei	Sheath is unstained with Giemsa, nuclei extend to tail tip	Small size, no sheath, in *M. perstans* nuclei extend to tail tip	Sheath is unstained with Giemsa, tail is anucleate

11B-H

Correct diagnosis: No Parasites Seen.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
No Parasites Seen	26/28	93	10/10	Correct
Parasites Seen	2	7	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that **No Parasites Seen** was the correct response (93 and 100%). Quality control examination of 4% of this sample showed erythrocytes of normal size and staining characteristics. Normal blood elements are present and exhibit typical staining characteristics. The overall staining quality is good.

11B-I

Correct diagnosis: *Trypanosoma brucei*.

Results of Participating Laboratories

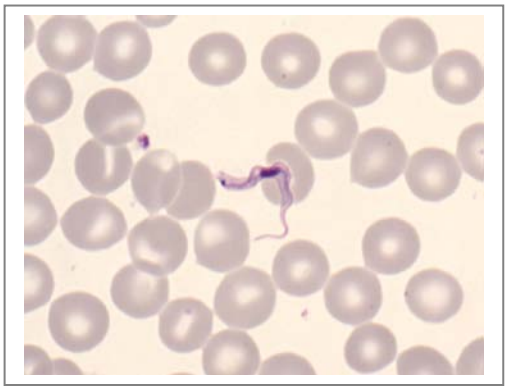
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Trypanosoma brucei	18/28	64	10/10	Correct
Parasites Seen	7	25	0	Correct
Trypanosoma cruzi	3	11	0	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that ***Trypanosoma brucei*** was the correct response (100%). Quality control examination of 4% of this sample showed parasites in every 5-6 100 X oil immersion fields. They have a central nucleus and a small posterior kinetoplast. The overall staining quality is good.

Diagnostic Characteristics

Trypanosoma brucei is the causative agent of African sleeping sickness. It is limited to the tse tse fly endemic area of Central Africa where it has caused serious economic and social problems.



Trypomastigotes are detected in the blood on thick and thin Giemsa stained smears. They measure 14-33 µm long and in some cases the undulating membrane and flagellum may be seen. The nucleus is located in the middle of the organism, and the kinetoplast, which surrounds the base of the flagellum, is located at the blunt posterior end. On a Giemsa stained smear the cytoplasm will stain blue and the nucleus and kinetoplast will stain red or purple. Trypomastigotes of *T. cruzi* are generally shorter and have more pointed posterior ends. They also have a much larger and more prominent kinetoplast, and often assume a C or U shape.

11B-J

Correct diagnosis: *Plasmodium falciparum.*

Results of Participating Laboratories

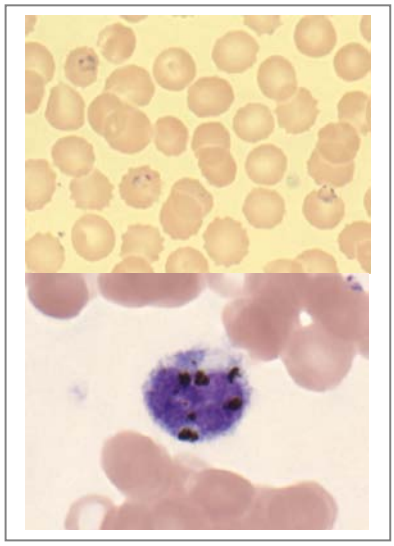
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium falciparum	19/28	68	10/10	**Correct**
Parasites seen	7	25	0	**Correct**
Plasmodium vivax	1	4	0	Incorrect
Babesia sp.	1	4	0	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that ***Plasmodium falciparum*** was the correct response (100%). Quality control examination of 4% of this sample showed parasites in every 100 X oil immersion field. Infected cells are not enlarged nor is there any visible stippling. The only stage seen was the ring stage trophozoite. The staining quality is good.

Diagnostic Characteristics

Plasmodium falciparum is one of the four species of *Plasmodium* know to infect humans. It causes the most dangerous and severe form of malaria and is always considered to be a medical emergency. Death may occur rapidly if proper treatment is not started immediately. Its distribution is limited to the tropics, primarily Africa and Asia.



P. falciparum invades all ages of RBCs and so the parasitemia can exceed 50%. The usual stages seen in the peripheral blood are rings and gametocytes. Schizogony occurs in the internal organs, so it is rare to seen other stages although they may be present in cases of severe malaria. The infected RBCs are not enlarged nor do they contain Schüffner's dots. The rings are generally small, and may have one or two chromatin dots. Appliqué forms, such as that seen in the infected cell on the lower right of the top image, are also characteristic. Gametocytes are rounded to banana- shaped and contain a single well defined chromatin and coarse rice-grain-like pigment.

Although *P. falciparum* rings somewhat resemble those of *Babesia* sp., there are many diagnostic criteria that can help to distinguish between them. One is the presence of pigment, a byproduct of hemoglobin digestion that is absent in *Babesia* but present in *Plasmodium* species. This pigment can be found in parasite cells, and also in white blood cells (lower image) that have consumed parasites or pigment granules.

Scoring Information

Distribution of Scores

Score	# of labs	% of labs
100	18/28	64
80-89	7	25
60-69	1	4
40-49	2	7

Answer Key

Sample	Correct Answer	Points
11B-F	*Plasmodium ovale*	20
11B-G	*Loa loa*	20
11B-H	No Parasites Seen	20
11B-I	*Trypanosoma brucei*	20
11B-J	*Plasmodium falciparum*	20

TOTAL POSSIBLE POINTS 100

Grading

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 www.phppo.cdc.gov. 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 finding parasites or ova 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:

$$\frac{\text{Number of correct responses by lab}}{\text{\# Correct Parasites Present + \# Lab's Incorrect Answers}} \times 100$$

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/WebGuide.pdf

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.