NEW YORK STATE
Parasitology Proficiency Testing Program

Blood Borne Parasites
01 June 2010

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 June 2010 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, 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.

10B-F

Correct diagnosis: *Brugia malayi*.

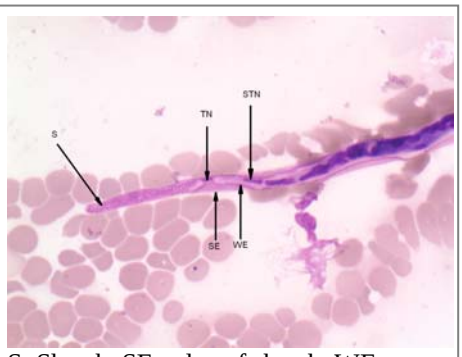
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Brugia malayi	8/20	40	8/10	**Correct**
Loa loa	7	35	0	Incorrect
Mansonella sp.	1	5	1	Incorrect
Wuchereria bancrofti	1	5	0	Incorrect
No Parasites Seen	3	15	1	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that ***Brugia malayi*** was the correct response (80%). Quality control examination of 4% of this sample showed an average of 3 parasites per slide. There is a pink staining sheath and terminal and subterminal nuclei in the tail tip. The overall staining quality is good.

Parasite Information and Diagnostic Characteristics



S: Sheath. SE: edge of sheath. WE: Edge of worm. TN: terminal nucleus. STN: second terminal nucleus. (Click to enlarge image.)

Brugia malayi is a filarid, which are worms that reside in the lymphatic system of humans and which are spread by insect bite. Adult female worms produce large numbers of larvae called *microfilariae* which can be detected in the peripheral blood. Diagnosticians should always scan the entire Giemsa-stained slide at low power to efficiently detect microfilarids; they are easily visible using a 10X objective.

Microfilariae of *Brugia malayi* range in size from 177-230 µm and have a **clearly visible pink sheath when stained with Giemsa stain**. *Wuchereria bancrofti* and *Loa loa* also have sheaths, but they do not stain with Giemsa; they are visible as a clear zone around the body of the larva. *Brugia malayi* is also characterized by the presence of two terminal nuclei, one of which is located in the tip of the tail. *Wuchereria bancrofti* has no nuclei in the tip of the tail and *Loa loa* has a continuous irregularly spaced row extending all the way to the tip.

10B-G

Correct diagnosis: *Plasmodium ovale.*

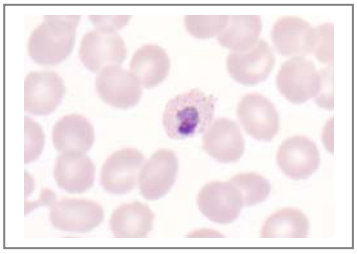
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium ovale	7/20	35	6/10	**Correct**
Plasmodium vivax	1	5	0	No Penalty
Plasmodium malariae	1	5	1	No Penalty
No Parasites Seen	11	50	3	No Penalty

Quality Control and Referee Information

Participating and referee laboratories failed to agree that ***Plasmodium ovale*** was the correct response. Quality control examination of 4% of this sample showed parasites in every 25-30 100 X oil immersion fields. The infected cells are somewhat enlarged and exhibit Schüffner's stippling. The cytoplasm of the parasite is compact and they have a large chromatin dot. The staining quality is good.

Parasite Information and Diagnostic Characteristics



Plasmodium ovale infections account for fewer than 5% of all malaria cases, and are relatively easy to confuse with *P. vivax* infections. *Plasmodium ovale* can be distinguished from other *Plasmodium* species by differences in the appearance of the infected red blood cell and parasite. The infected cells are usually enlarged up to 1 ½ x their normal diameter, fimbriate (having a "fringed" border), and have Schüffner's stippling. *P. vivax*-infected RBC's are even larger (up to 2x normal diameter) and less rounded in shape. *P. malariae*-infected RBC's are never enlarged, and are often actually smaller than uninfected RBC's. The cytoplasm of *P. ovale* trophozoites is usually less amoeboid than then that of *P. vivax,* and the schizonts have 4-12 merozoites compared to 12-24 for *P. vivax*. The chromatin of *P. ovale* is usually very pronounced and the pigment is coarse.

Remember that at least 200-300 100X oil immersion fields should be examined before calling a specimen negative. While the parasitemia in this sample was not high, 8 infected RBC's would have been visible on these slides for every 200 fields examined.

10B-H

Correct diagnosis: No Parasites Seen.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
No Parasites Seen	20/20	100	10/10	**Correct**

Quality Control and Referee Information

Participating and referee laboratories agreed that **No Parasites Seen** was the correct response (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.

10B-I

Correct diagnosis: *Loa loa*.

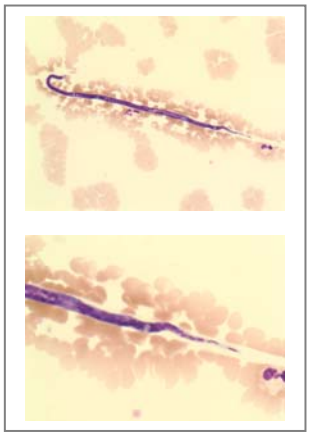
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Loa loa	6/20	30	5/10	**Correct**
Mansonella sp.	10	50	5	No Penalty
No Parasites Seen	4	20	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories failed to agree that ***Loa loa*** was the correct response but did agree that there were parasites present (80 and 100%). Quality control examination of 4% of this sample showed greater than 20 organisms per slide. The parasites have an unstained sheath which can be difficult to see and nuclei extending in a single row all the way to the tip of the tail.

Parasite Information and Diagnostic Characteristics



Loa loa, also called the African eye worm, is a filarid like *Brugia malayi* (10B-F). *Loa loa* microfilariae are sheathed and measure between 230-250 µm. They have nuclei that extend all the way to the tip of the tail, unlike the two other sheathed species *Wucheria bancrofti* and *Brugia malayi.* Although the sheath does not stain with Giemsa, it is still visible as a clear area around the body of the worm, as shown in the images on the left. As noted for sample 10B-F, the best way to detect microfilarids is to scan the entire slide at 10X power; the dark-staining nuclei and large size of the microfilarids make them very prominent against the field of red blood cells.

10B-J

Correct diagnosis: *Plasmodium falciparum.*

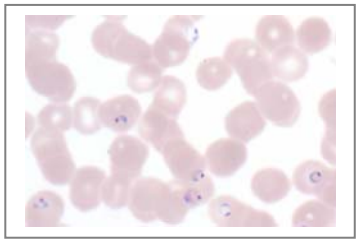
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium falciparum	20/20	100	10/10	**Correct**

Quality Control and Referee Information

Participating and 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. The infected cells are not enlarged. There are many rings, some with double chromatin, and appliqué forms. The staining quality is good.

Parasite Information and Diagnostic Characteristics



Plasmodium falciparum causes the most dangerous and severe form of human malaria and is always considered to be a medical emergency. *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 see other stages in peripheral blood, 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 usually smaller and more delicate than those of other *Plasmodium* species, and may have one or two chromatin dots. Appliqué forms (which look as if they are bulging out from the side of the RBC) are also characteristic. While *Babesia* species also produce small ring forms, these are often vacuolated, which *P. falciparum* rings are not. Gametocytes of *P. falciparum* are very distinctive: they are rounded to banana- shaped and contain a single well defined chromatin dot and coarse rice-grain like pigment. However, most patient samples will contain few or no gametocytes; ring forms are much more common.

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	Speceis 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

Scoring Information

Distribution of Scores

Score	# of labs	% of labs
100	8/20	40
80	8	40
60	4	20

Answer Key

Sample	Correct Answer	Points
10B-F	*Brugia malayi*	20
10B-G	*Plasmodium ovale*	20
10B-H	No Parasites Seen	20
10B-I	*Loa loa*	20
10B-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 5, 2010.** You are responsible for notifying the New York State Parasitology Unit **before October 12, 2010** if you do not receive your test. Proficiency test results must be electronically submitted through EPTRS by **October 19, 2010** or you will receive a zero. These requirements are clearly stated in your NYS Proficiency Testing Handbook provided by the NYS Clinical Laboratory Evaluation Program, and can be accessed via the Internet at: http://www.wadsworth.org/labcert/clep/ProgramGuide/WebGuide.pdf

News and Notes

In conjunction with the Northeast Regional Meeting of the American Society for Microbiology Meeting on November 9 and 10 in Albany, the Parasitology Laboratory will be conducting a half-day workshop called “Separated at Birth”. This workshop will feature parasites that are morphologically similar and can be challenging to differentiate.