NEW YORK STATE

Parasitology Proficiency Testing Program

Blood Borne Parasites
01 February 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 February 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, 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-A

Correct diagnosis: *Trypanosoma cruzi*.

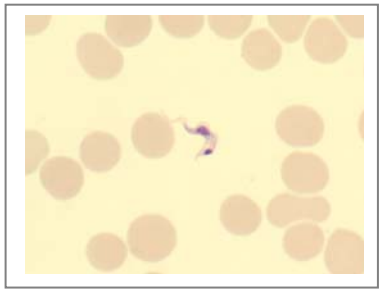
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Trypanosoma cruzi	18/19	95	10/10	**Correct**
Trypanosoma gambiense	1	5	0	Incorrect

Quality Control and Referee Information

Participating and referee laboratories agreed that ***Trypanosoma cruzi*** was the correct response (95 and 100%). Quality control examination of 4% of this sample showed parasites in every 4-5 100 X oil immersion fields. The organisms have a central nucleus and a large posterior kinetoplast. The overall staining quality is good.

Diagnostic Characteristics



Trypanosoma cruzi is the causative agent of Chagas disease, which is a major health problem in Latin America. The organism is transmitted by reduviid insects. Trypomastigotes of *T. cruzi* are detected in the blood on thin and thick smears. They measure approximately 20 µm and usually are C or U shaped. The nucleus is located in the middle of the organism and a large kinetoplast (which contains the ribosomal DNA) is located at the posterior end. A flagellum arises from the kinetoplastand follows the undulating membrane to the anterior end where it projects as a free flagellum. On Giemsa stained smears the cytoplasm stains blueish 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.* It should be noted that the subspecies of *Trypanosoma brucei* (*T. brucei gambiense* and *T. brucei rhodesiense*) cannot be distinguished by Giemsa stain, and therefore these names should not be used for reporting results.

11B-B

Correct diagnosis: *Brugia malayi.*

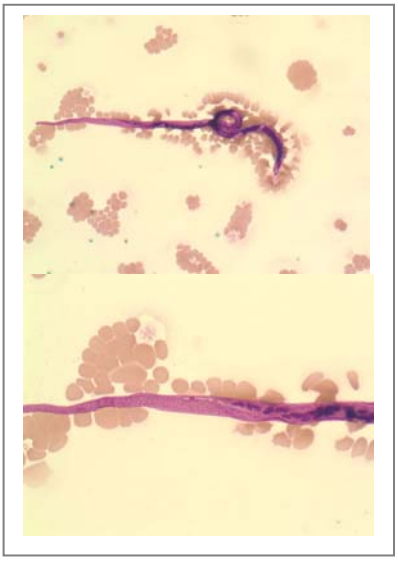
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Brugia malayi	9/19	47	9/10	**Correct**
Loa loa	3	16	1	Incorrect
Mansonella sp.	3	16	0	Incorrect
Wuchereria bancrofti	2	11	0	Incorrect
No Parasites Seen	2	11	0	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that ***Brugia malayi*** was the correct response (90%). Quality control examination of 4% of this sample showed an average of 10 organisms per slide. The parasites have a stained sheath and nuclei extending to the tail tip. The staining quality is good.

Diagnostic Characteristics



Brugia malayi is an arthropod-borne worm that resides in the lymphatic system of humans. Infection is spread by the arthropod intermediate host, in this case the mosquito. Adult female worms produce large numbers of sheathed larvae called microfilariae which can be detected in the peripheral blood.

Microfilariae of *B. 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* microfilariae also have sheaths, but they are not well stained with Giemsa and therefore may appear as a clear area surrounding the body of the filarid. *Mansonella* species lack a sheath. Another useful diagnostic characteristic is the distribution of dark-staining nuclei in the tail tip. *Brugia malayi* has two terminal nuclei, the second 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 row extending all the way to the tip.

An annotated image of the tailtip of B. malayi can be found in the June 2010 critique: http://www.wadsworth.org/parasitology/pdf/Jun_10_BBPO.pdf

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-C

Correct diagnosis: *Babesia* sp..

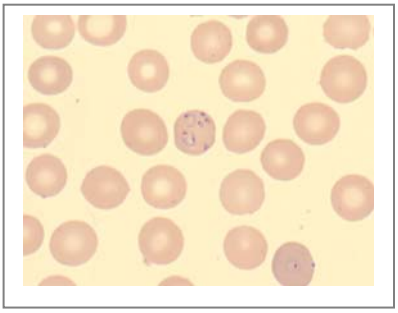
Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Babesia sp.	12/19	63	9/10	**Correct**
Plasmodium falciparum	7	37	1	Incorrect

Quality Control and Referee Information

Referee laboratories agreed that ***Babesia* sp.** was the correct response (90%). Quality control examination of 4% of this sample showed multiple parasites in every 100 X oil immersion field. There are multiple ring stage parasites per infected red blood cell and the parasitemia is quite high. Infected cells are not enlarged and no pigment is present. The staining quality is good.

Diagnostic Characteristics



***Babesia* sp.** has a widespread distribution, including several counties in New York State and other Northeastern states. Parasites are transmitted by several species of ticks, often *Ixodes scapularis* in the Northeast. Like their relatives the malaria parasites, *Babesia* infects red blood cells. In a Giemsa-stained thin smear, they appear as small, pleomorphic rings which can be confused with the early stage of *Plasmodium falciparum*. Infected cells are not enlarged and do not exhibit stippling or Mauer's dots, but they may be multiply infected. No other intraerythrocytic stages are ever seen and no pigment is ever present. Occasionally tetrads (a "Maltese cross"-like arrangement of four parasites) may be seen in the red blood cells. It is also fairly common for extraerythrocytic parasites to be observed.

11B-D

Correct diagnosis: No Parasites Seen.

Results of Participating Laboratories

Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
No Parasites Seen	19/19	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. The overall staining quality is good.

11B-E

Correct diagnosis: *Plasmodium vivax.*

Results of Participating Laboratories

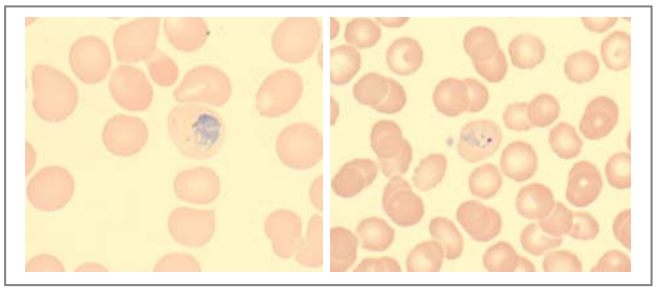
Organism reported	# of labs reporting	% of labs reporting	Referee results	Status
Plasmodium vivax	8/19	42	5/10	**Unauthenticated**
Plasmodium malariae	6	32	3	No Penalty
Plasmodium ovale	4	21	2	No Penalty
Plasmodium falciparum	1	5	0	No Penalty

Quality Control and Referee Information

Participating and referee laboratories failed to agree that ***Plasmodium vivax*** was the correct response (42 and 50%). Quality control examination of 4% of this sample showed parasites in every 8-10 100 X oil immersion fields. Most of the infected cells are enlarged, as shown in the image, which excludes *P. falciparum* and *P. malariae* from consideration. There is no Schüffner's stippling and the pigment is scattered and fine. The staining quality is good with infected cells staining paler than uninfected cells.

Diagnostic Characteristics

Plasmodium vivax is the most common species of malaria to infect humans. It may account for as much as 80% of all malaria cases. It also has the widest distribution. Infected red cells are usually enlarged and stain paler than uninfected ones. They may also contain Schüffner's dots. The trophozoites are generally amoeboid and have a large chromatin dot. Occasionally cells will contain more than one parasite. Mature schizonts contain 12-24 merozoites and gametocytes are round and fill the entire cell. Pigment is fine and scattered.



Scoring Information

Answer Key

Sample	Correct Answer	Points
11B-A	*Trypanosoma cruzi*	20
11B-B	*Brugia malayi*	20
11B-C	*Babesia* sp.	20
11B-D	No Parasites Seen	20
11B-E	*Plasmodium vivax* (unauthenticated)	20

TOTAL POSSIBLE POINTS 100

Distribution of Scores

Score	# of labs	% of labs
100	7/19	37
80	6	32
60	6	32

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 **May 17, 2011.** You are responsible for notifying us **before May 24, 2011** if you do not receive your samples. Proficiency test results must be electronically submitted through EPTRS by **May 31, 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.