# NEW YORK STATE

*Parasitology Proficiency Testing Program*

# Blood Smears Only
## 3 February 2015

The purpose of the New York State Proficiency Testing Program in the category of Parasitology - Blood Smears Only is to monitor the performance of laboratories that detect and identify parasites in blood smears. Below please find the results for the February 2015 proficiency test in Blood Smears Only.

This category is divided into two sub-categories. **Parasite Identification** is intended for labs that identify parasites and report them to the genus or species level on patient reports. **Parasite Screen** is intended for labs that report "Parasites Seen" but do not identify organisms on patient reports. Participants in both sub-categories examine the same samples, however the scoring criteria for the two sub-categories are different. When reading this critique, please ensure that you are comparing your performance to other laboratories in your sub-category.

# Sample Preparation and Quality Control

All slides used in this test were prepared and stained by a commercial source. Samples of each test specimen were selected at random by the Parasitology Laboratory at the Wadsworth Center, NYS DOH, and were assayed for quality and confirmation of contents. The supplying vendor also conducted extensive quality control tests and a detailed quality control report was submitted to the Parasitology Laboratory for inspection and verification. Samples were authenticated by 80% of participating laboratories and/or referee laboratories.

# 15B-A

Correct Identification: *Loa loa*

## *Results of Participating Laboratories Who Perform Parasite Identification*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| *Loa loa* | 10/21 | 48 | 6/10 | **Correct*** |
| *Mansonella* sp. | 10 | 48 | 4 | **No Penalty*** |
| *Plasmodium* sp. | 1 | 4 | 0 | **Incorrect** |

* Credit was given for *Loa loa* and *Mansonella* species as this sample was authenticated only as microfilaria.

## *Results of Participating Laboratories Who Perform Parasite Screen*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| Parasites Seen | 6/6 | 100 | 10/10 | **Correct** |

## *Quality Control and Referee Information*

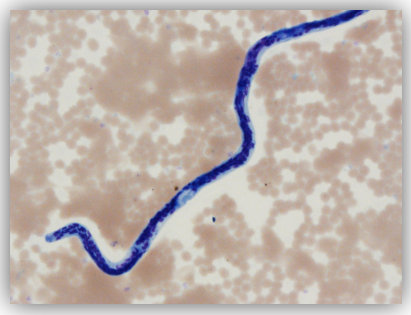
This sample contained microfilariae of ***Loa loa***, the African eye worm. Because participating and referee laboratories were split between identification as *Mansonella* and *Loa loa* the specimen was authenticated only as microfilaria. Quality control examination of 4% of the slides for this sample showed an average of > 4 organisms per slide. The overall staining quality was good.

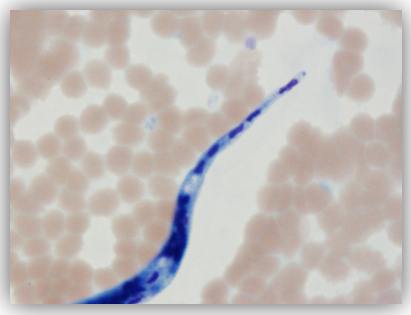
## *Diagnostic Characteristics*

Microfilariae of *Loa loa* are sheathed, measure between 250-300 μm, and have nuclei that are irregularly spaced but extend to the tip of the tail. However, the sheath of *Loa loa* does not always stain well with Giemsa and the microfilaria can lose their sheath. This situation is likely to have lead to misidentification as *Mansonella*. *Mansonella perstans* is also found in the blood and has nuclei that extend to the tip of the tail, though they have no sheath and the nuclei are more regularly spaced.

In the initial analysis of blood smears, it is critical to scan the slide under low (10-20X) power to screen for microfilaria. The appearance of the short headspace and irregularly spaced nuclei extending to the tip of the tail are important characteristics for identification.

Both *Loa loa* and *Mansonella perstans* are found in Africa, though *M. perstans* is also found in South America. Deer or mango flies transmit *Loa loa* while midges transmit *Mansonella* species. In both cases the larvae are deposited into the bite wound. *Loa loa* is most likely to be found in peripheral blood between 10 AM and 2 PM, and is the only filarid known to have diurnal periodicity.





# 15B-B

Correct Identification: *Trypanosoma brucei*

## *Results of Participating Laboratories Who Perform Parasite Identification*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| *Trypanosoma brucei* | 18/20 | 90 | 9/10 | **Correct** |
| *Trypanosoma cruzi* | 2 | 10 | 1 | **Incorrect** |

## *Results of Participating Laboratories Who Perform Parasite Screen*

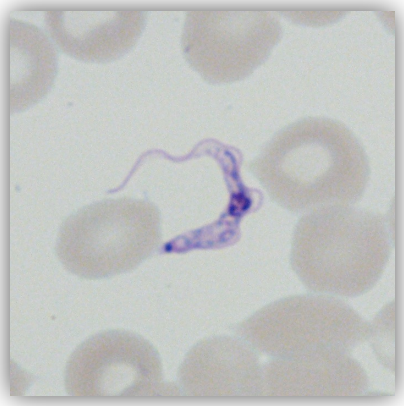
| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| Parasites Seen | 6/6 | 100 | 10/10 | **Correct** |

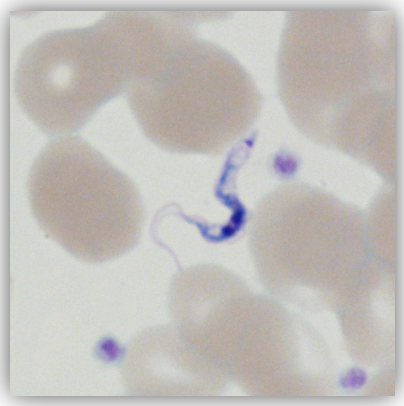
## *Quality Control and Referee Information*

Participating and referee laboratories agreed that ***Trypanosoma brucei*** was the correct response (90 and 90% respectively). Quality control examination of 4% of the slides for this sample showed an average of 9 organisms in every 40X field. Staining quality was good.

## *Diagnostic Characteristics*

This blood slide contained trypomastigotes of *Trypanosoma brucei* the causative agent of African sleeping sickness, also know as human African trypanosomiasis (HAT). This flagellated protozoa is most similar to *T. cruzi*, which causes Chagas' disease and is found primarily in South and Central America. The major morphological difference is the large kinetoplast (mitochondrial DNA) observed in *T. cruzi* compared to *T. brucei*.
African trypanosomiasis is limited to the tse-tse fly endemic area of Sub-Saharan Africa, where it has caused serious economic and social problems. If left untreated the infection is fatal.





# 15B-C

Correct Identification: *Plasmodium ovale*

## *Results of Participating Laboratories Who Perform Parasite Identification*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| *Plasmodium malariae* | 11/21 | 52 | 8/10 | **Accepted*** |
| *Plasmodium vivax* | 6 | 29 | 0 | **Accepted*** |
| *Plasmodium ovale* | 4 | 19 | 2 | **Intended Answer*** |

* Credit was given for all *Plasmodium* species as this sample was authenticated only as *Plasmodium* species.

## *Results of Participating Laboratories Who Perform Parasite Screen*

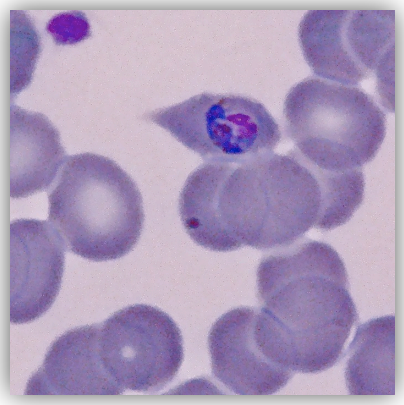
| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| Parasites Seen | 6/6 | 100 | 10/10 | **Correct** |

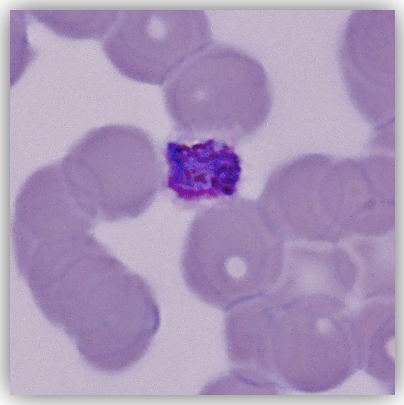
## *Quality Control and Referee Information*

This sample contained ***Plasmodium ovale***. Because participating and referee laboratories were split between identification as *Plasmodium ovale*, *P. malariae*, and *P. vivax* the specimen was authenticated only as *Plasmodium* species. Quality control examination of 4% of the slides for this sample showed an average of 1-5 organisms in every 40X field. Staining quality was good. Real-time PCR was also performed on this sample and *Plasmodium ovale* was confirmed as the correct identification.

## *Diagnostic Characteristics*

This specimen contained early and late trophozoites as well as gametocytes. *Plasmodium ovale* is most similar to *P. vivax* in morphology. Infected red blood cells are enlarged (1.5 - 2X) for *P. vivax* and normal to 1.5X for *P. ovale*. Cells may be fimbriated and may have Schüffner's stippling. *P. vivax* infected cells are amoeboid and the cytoplasm is fragmented. *P. ovale* infected cells are often oval in shape and the cytoplasm is more compact. These distinguishing features are very different than characteristics observed for *P. malariae.* Cells infected with *P. malariae* are normal in size or smaller than uninfected cells with no stippling and are not fimbriated.





# 15B-D

Correct Identification: No Parasites Seen

*Results of Participating Laboratories Who Perform Parasite Identification*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| No Parasites Seen | 21/21 | 100 | 10/10 | **Correct** |

*Results of Participating Laboratories Who Perform Parasite Screen*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| No Parasites Seen | 6/6 | 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 slides for this sample showed erythrocytes of normal size and staining characteristics. Normal blood elements are present and exhibit typical staining characteristics. The overall staining quality was good.

# 15B-E

Correct Identification: *Plasmodium falciparum*

## *Results of Participating Laboratories Who Perform Parasite Identification*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| *Plasmodium falciparum* | 20/22 | 91 | 8/10 | **Correct** |
| *Babesia* sp. | 2 | 9 | 2/10 | **Incorrect** |

## *Results of Participating Laboratories Who Perform Parasite Screen*

| Organism reported | # of labs reporting | % of labs reporting | Referee results | Status |
|---|---|---|---|---|
| Parasites Seen | 5/5 | 100 | 10/10 | **Correct** |

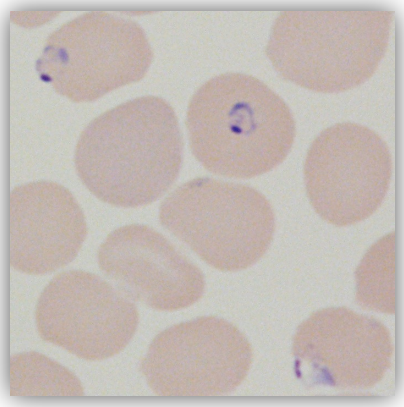
## *Quality Control and Referee Information*

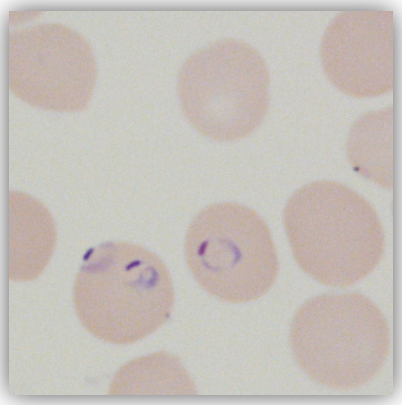
Participating and referee laboratories agreed that ***Plasmodium falciparum*** was the correct response (91 and 80% respectively). Quality control examination of 4% of the slides for this sample showed numerous trophozoites, an average of 10 organisms per 100X oil immersion field. Staining quality was good.

## *Diagnostic Characteristics*

***Plasmodium falciparum*** is generally causes the most severe malaria as the parasite invades all ages of red blood cells and has reproductive cycle of 36-48 hours. As a result the parasitemia can exceed 30%. For *P. falciparum*, the stage seen in peripheral blood is early trophozoites, or rings. Both cells with more than one ring and applique forms were present in this specimen as is common in *P. falciparum* infections. 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*.





# Scoring Information

## *Distribution of Scores*

| Score | # of labs | % of labs |
|---|---|---|
| 100 | 22 | 81 |
| 90-99 | 0 | 0 |
| 80-89 | 4 | 15 |
| 70-79 | 1 | 4 |
| 60-69 | 0 | 0 |
| 0-59 | 0 | 0 |

## *Answer Key*

| Sample | Correct Answer |
|---|---|
| **15B-A** | *Loa loa* * |
| **15B-B** | *Trypanosoma brucei* |
| **15B-C** | *Plasmodium ovale* ** |
| **15B-D** | No Parasites Seen |
| **15B-E** | *Plasmodium falciparum* |

* This sample was authenticated as microfilaria.
** This sample was authenticated as *Plasmodium* species.

## *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% but less than 80% of the participating laboratories **or** referees are "Unauthenticated", and are not considered for grading.

Credit is given according to the formula:

[# of Correct Responses / (# of Correct Responses + # of Incorrect Responses)] X 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:

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

# Important Reminders

The next Parasitology Proficiency Test is scheduled for **May 19, 2015.** Participating labs will need to notify us **before May 26, 2015** if the samples are not received. Proficiency test results must be electronically submitted through EPTRS by **June 3, 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/CLEPGUIDE-March2015.pdf