

# Malaria rapid diagnostic test and Giemsa – stained peripheral blood smear discrepancies in the diagnosis of *Plasmodium ovale* infection in New England

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## ABSTRACT

Malaria is a serious mosquito-borne parasitic disease and is one of the most significant causes of death worldwide. In this report we discuss a case of a 26-year-old female healthcare worker who presented to the emergency department with signs and symptoms of persistent fevers, chills, headaches, nausea, and malaise. The patient's history was obtained and several laboratory tests were performed leading to the diagnosis of malaria caused by *Plasmodium ovale*. The rapid diagnostic test for malaria was negative, and the Giemsa – stained peripheral blood smears were positive for the presence of malarial parasites. Images of the peripheral blood smears were sent to the Centers for Disease Control and Prevention, which confirmed the presence of *Plasmodium ovale*. We also report 5 other false negative RDTs for *P. ovale* in our institution in 2016, and the performance of RDTs for all malaria cases during that year. This case demonstrates the importance of obtaining multiple pieces of laboratory data in order to accurately diagnose a patient with malaria, illustrates that evaluation of Giemsa – stained peripheral blood smears is still the gold standard in the laboratory diagnosis of malaria, and emphasizes the importance for clinical laboratory scientists to maintain maximum competency in peripheral blood smear evaluation for bloodborne parasites.

**ABBREVIATIONS:** CBC - Complete blood count, RDT - Rapid Diagnostic Test, RBCs - Red blood cells, CDC - Centers for Disease Control and Prevention, AST - Aspartate Aminotransferase, ALT - Alanine Aminotransferase

**INDEX TERMS:** Malaria, malaria rapid diagnostic tests, malaria microscopy, *Plasmodium ovale*

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## CASE REPORT

A 26-year-old female presented to the emergency department after a week of intermittent fever, chills, headaches, nausea, and malaise. Her symptoms were waxing and waning with the highest temperature up to 39.4 °C. She denied abdominal pain, diarrhea, vomiting, dysuria, hematuria, cough, or skin rashes. Abdominal examination of the patient revealed possible splenomegaly. She was seen by her primary care physician three days after her symptom onset and was told her symptoms were likely of viral origin. Influenza and Monospot tests were negative at that time. She attempted to take ibuprofen for the fever, but her symptoms were worsening. Her history was significant for traveling to Africa where she was as a healthcare worker for two years in Tanzania and Zambia. She obtained appropriate immunizations prior to travel and took anti-malarial medications during the first half of her stay, but failed to complete the treatment during the second half. The patient denied any illness during that period. She had exposure to tuberculosis and HIV patients, but her HIV, tuberculosis, and hepatitis panels

remained negative after returning to the United States.

The laboratory results ordered included but were not limited to a CBC, peripheral blood smears, electrolytes, coagulation tests, and urinalysis (Table 1). The CBC results for the patient suggested severe hemolytic anemia with pancytopenia. Her *Anaplasma*, *Ehrlichia*, *Babesia*, and Lyme antibody tests were all negative. The patient was tested for malaria using the BinaxNOW Malaria Test. This RDT is an *in vitro* immunochromatographic assay for the qualitative identification of *Plasmodium* antigens.<sup>1</sup> The results of this test were negative. However, Giemsa – stained thick and thin peripheral blood smears demonstrated the presence of ring form trophozoites. The percent parasitemia was 1.2%. The laboratory scientists had evidence to believe that the parasite was *Plasmodium ovale* based on the morphological characteristics that were displayed on the peripheral blood smears. Characteristic of *P. ovale*, the RBCs were slightly enlarged, fimbriated, and contained Schüffner's dots (Figures 1 and 2). The presence of Schüffner's dots

allowed the laboratory scientists to rule out *P. falciparum* and *P. malariae* since they lack these structures. RBCs infected with *P. vivax* can also demonstrate Schüffner's dots, but it was ruled out because the RBCs that were infected were fimbriated, which is a unique characteristic of *P. ovale* (Figures 1 and 2). Also, RBCs infected with *P. vivax* tend to be more enlarged than those infected with *P. ovale*.

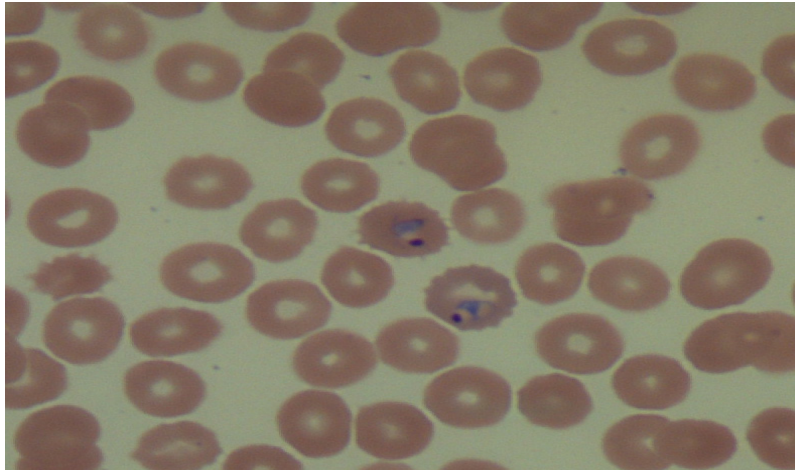
The laboratory scientists believed that the inconsistency between the RDT and the peripheral blood smears could have been due the inability of the RDT to detect a parasitemia of 1.2%. Upon discussion between the laboratory scientists and the pathologists of the laboratory medicine department, several peripheral blood smear images were sent to the CDC. Specialists at the CDC confirmed the parasites to be *P. ovale*, the majority of which were in the ring-form trophozoite stage. Some schizonts were also detected (Figure 3).

The patient was administered Coartem for treatment,

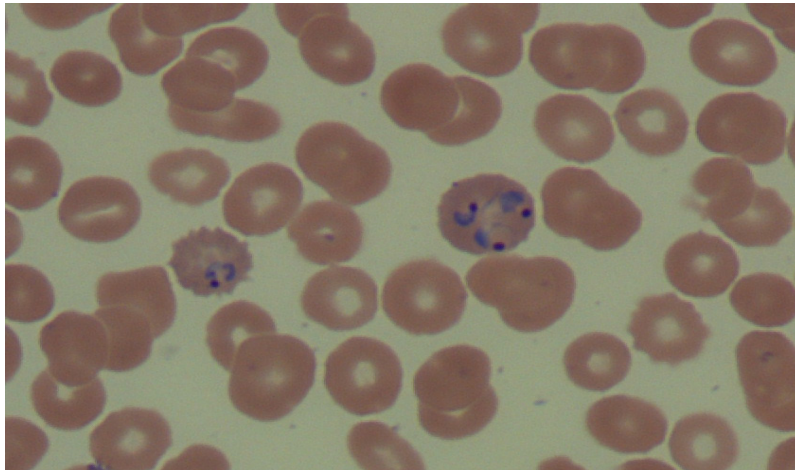
**Table 1.** Report of relevant laboratory testing at time of admission

Test	Patient Result	Reference Range for Adult Female
<b>Hematology</b>		
Red blood cell count	3.53 x 10 <sup>12</sup> /L	*L 4.00-5.20 x 10 <sup>12</sup> /L
White blood cell count	1.9 x 10 <sup>9</sup> /L	*L 4.0-11.0 x 10 <sup>9</sup> /L
Hemoglobin	8.7 g/L	*L 11.8-16.0 g/L
Hematocrit	27.3%	*L 36-44%
Platelet count	26 x 10 <sup>9</sup> /L	*L 150-450 x 10 <sup>9</sup> /L
D-dimer	888.74 nmol/L (2,685 ng/mL) *H	<80.43 nmol/L (<243 ng/mL)
<b>Chemistry</b>		
Sodium	136 mmol/L	136-142 mmol/L
Glucose	6.60 mmol/L	*H 3.9-6.1 mmol/L
Total calcium	8.8 mmol/L	*H 2.1-2.8 mmol/L
Total protein	0.05 g/L	*slightly L 0.06-0.08 g/L
ALT	27 IU/L	*H 8-20 IU/L
AST	35 IU/L	*H 8-20 IU/L
Total Bilirubin	22.24 umol/L	*H 5.0-21.0 umol/L
Direct Bilirubin	8.55 umol/L	*H 1.7-5.1 umol/L
<b>Urinalysis</b>		
Clarity	Cloudy	Clear
Color	Yellow	Pale yellow, yellow
Protein	1+	*H Negative
Blood	1+	*H Negative
<b>Microbiology</b>		
Comprehensive respiratory panel	Negative	Negative
Blood culture (anaerobic and aerobic)	Negative	Negative
Urine culture	Negative	Negative
Antibody for <i>Anaplasma</i> , <i>Erlichia</i> , <i>Babesia</i> , Lyme	Negative	Negative

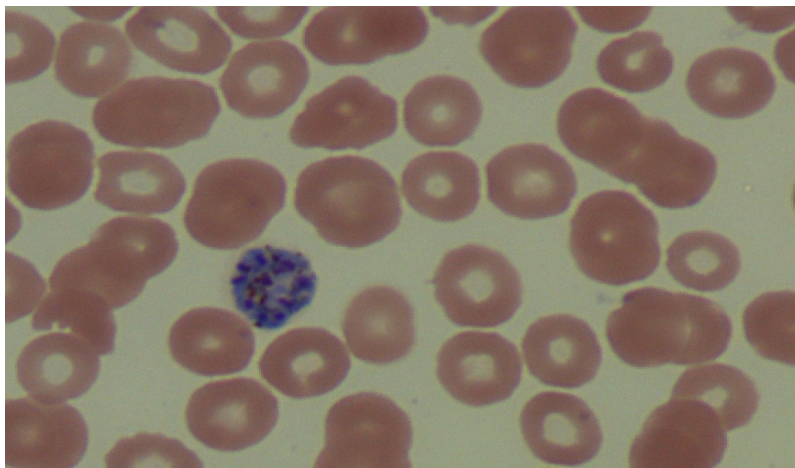
Low (L), High (H), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST).



**Figure 1.** Peripheral blood smear from the patient demonstrating two fimbriated erythrocytes with ring form trophozoites of *P. ovale*. Giemsa stain. x1000.



**Figure 2.** Peripheral blood smear from the patient demonstrating enlarged erythrocyte with ring form trophozoites of *P. ovale*. Giemsa stain. x1000.



**Figure 3.** Peripheral blood smear from the patient demonstrating a schizont form of *P. ovale*. Giemsa stain. x1000.

which is a combination drug of the two antimalarial agents artemether and lumefantrine. The exact mechanism of action of these agents is not well understood, however, studies have demonstrated that they inhibit nucleic acid and protein synthesis of the parasites.<sup>2</sup> In addition to Coartem, the patient was further treated with primaquine phosphate, which targets the hypnozoite stage of *P. ovale* and *P. vivax*. This treatment is necessary to prevent relapse of the disease due to sequestered parasites in the liver.<sup>3</sup>

It is important to note that in 2016, 178 specimens were submitted for malaria testing using the BinaxNOW RDT (Table 2). There were 151 tests (84.8%) that were true negatives (negative RDT, negative blood smear for *Plasmodium* spp.). There were 20 (11.2%) true positives (positive RDT, positive blood smear for *Plasmodium* spp.), 6 (3.4%) false negatives (negative RDT, positive blood smear for *Plasmodium* spp.), and 1 (0.6%) false positive (positive RDT, negative blood smear for *Plasmodium* spp.). The CDC confirmed that the 5 of the 6 false negatives were caused by *P. ovale*. One of those 6 is still awaiting confirmation from the CDC as to whether the organism is *P. vivax*, or *P. ovale*. Therefore, all of the false negative RDTs most likely were due to infection with *P. ovale*. Percent parasitemia was low in

these patients (Table 3). Only one BinaxNOW test was positive in a patient with *P. ovale* infection. This data indicates an overall diagnostic sensitivity of 76.9%, diagnostic specificity of 99.3%, a positive predictive value of 95.2%, and a negative predictive value of 96.2%.

## DISCUSSION

The World Health Organization states that over three billion people are at risk for malaria. In 2015, Sub-Saharan Africa accounted for 88% of the global malaria cases and 90% of deaths caused by malaria.<sup>4</sup> In the United States, roughly 1,500 cases per year are diagnosed. Most of these cases involve travelers, especially healthcare workers, and immigrants from malaria endemic countries.<sup>5</sup> The etiology of malaria is a protozoan parasite of the genus *Plasmodium*. There are four main species of *Plasmodium* that cause disease in humans. These four are *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.<sup>6</sup> *P. ovale* is found predominately throughout sub-Saharan Africa.<sup>6</sup> It is also found in the Western Pacific islands.<sup>7</sup> In Tanzania, *P. ovale* accounts for greater than 10% of *Plasmodium* species; Zambia it accounts for up to 5% of *Plasmodium* species.<sup>8,9</sup>

The life cycle of malaria caused by *P. ovale*, as well as the other *Plasmodium* species involves two hosts. During the

**Table 2.** BinaxNOW Rapid Test Results vs. Peripheral Blood Smears of 178 samples in 2016

Lines Present on BinaxNOW Test	Negative Blood Parasite Smear	Positive Smear for <i>P. ovale</i>	Positive Smear for <i>P. falciparum</i>	Positive Smear for <i>Plasmodium</i> species not <i>falciparum</i>	Positive Smear for <i>Plasmodium</i> species, either <i>P. ovale</i> & <i>P. vivax</i> (pending confirmation from CDC)	Positive Smear for <i>Plasmodium</i> species (pending confirmation from CDC)	Positive Smear for <i>Babesia</i> spp.
No Lines	149/178 (83.7%)	5/178 (2.8%)			1/178 (0.6%)		2/178 (1.1%)
T1 only	1/178 (0.6%)		7/178 (3.9%)				
T2 only				1/178 (0.6%)			
T1+T2		1/178 (0.6%)	10/178 (5.6%)			1/178 (0.6%)	

### Interpretation of BinaxNOW Results<sup>1</sup>

T1: "Positive for *P. falciparum* antigen only. Confirmation by smear and parasitemia to follow."

T2: "Positive for malaria protein antigen representing either *P. vivax*, *P. ovale*, *P. malariae* or a mix of these. Differentiation of the species is not possible with this test. Confirmation by smear and parasitemia to follow."

T1 + T2: "Positive for *P. falciparum*. In some cases this may represent a mix of *P. falciparum* with non-*falciparum* species. Differentiation between a *P. falciparum* only infection and a mixed infection is not possible with this test. Confirmation by smear and parasitemia to follow."

No T1 or T2 Lines: "Presumptive negative for malaria antigens. Confirmation by smear to follow."



**Table 3.** Parasitemia Levels for Positive Blood Smears

Samples negative for malaria antigens with positive <i>P. ovale</i> smear (5/178)	Parasitemia (%)
1	<0.1
2	0.1
3	0.1
4	0.7
5	1.2
Sample negative for malaria antigens with positive <i>P. ovale</i> or <i>P. vivax</i> smear (pending confirmation from CDC) (1/178)	Parasitemia (%)
1	< 0.1
Samples positive for T1 only with positive <i>P. falciparum</i> smear (7/178)	Parasitemia (%)
1	<0.1
2	0.1
3	0.1
4	0.1
5	0.2
6	0.2
7	0.3
Sample positive for T2 only with positive <i>Plasmodium</i> species not <i>falciparum</i> smear (1/178)	Parasitemia (%)
1	0.3
Samples positive for T1 & T2 with positive smear for <i>P. falciparum</i> (10/178)	Parasitemia (%)
1	0.3
2	0.8
3	0.9
4	0.9
5	0.9
6	1.3
7	1.3
8	2.0
9	8.3
10	10.7
Sample positive for T1&T2 with positive smear for <i>P. ovale</i> (1/178)	Parasitemia (%)
1	0.1
Sample positive for T1&T2 with positive smear for <i>Plasmodium</i> species (pending species confirmation from the CDC) (1/178)	Parasitemia (%)
1	0.9
Sample negative for malaria antigens with positive smear for <i>Babesia</i> spp. (2/178)	Parasitemia (%)
1	0.2
2	0.9

process of a blood meal, a female *Anopheles* mosquito introduces sporozoites into a human host. Sporozoites invade the human liver, where they undergo asexual reproduction. This stage is known as exo-erythrocytic schizogony. During this stage, the organisms mature into schizonts, which eventually burst and release merozoites. There is a dormant stage with *P. ovale* and *P. vivax* infections in which the organism remains in the hepatocytes as hypnozoites. Hypnozoites can cause relapses by entering the bloodstream weeks, months, and even years later in some cases. Next, merozoites invade erythrocytes and undergo asexual reproduction. This is known as the erythrocytic schizogony stage. The organism develops into a ring-form trophozoite stage that matures into a schizont. The schizont eventually bursts, releasing merozoites, which will infect more erythrocytes, leading to increased hemolysis. Some parasites can differentiate into female and male gametocytes within the erythrocyte. These gametocytes, when taken up by the *Anopheles* mosquito undergo sexual reproduction (sporogonic cycle), with fusion of gametes and eventual migration of sporozoites to the mosquito salivary glands.<sup>6</sup>

The life cycle of *Plasmodium* spp. is important to understand because it is during the erythrocytic schizogony stage of the parasite's life cycle that the first signs and symptoms of malaria manifest. Fever is one of the most representative signs of malaria and occurs at different times due to maturation of schizonts at various time periods for each *Plasmodium* species. The schizonts of *P. vivax* and *P. ovale* mature every 48 hours; therefore the fever is tertian, occurring every two days. The malarial paroxysm consists of three stages. Chills characterize the first stage. The second stage is characterized by high fever with warm dry skin and accompanying headache, malaise, nausea, and vomiting. Profuse sweating occurs in the last stage, which causes the body temperature to decrease.<sup>10</sup>

Malaria caused by *P. falciparum* is more severe and rapidly progressive due to infected RBCs sticking to the walls of vessels of the microcirculation, causing blockage of blood flow with resulting ischemia of the affected tissue. Anemia is common in all malaria cases due to intravascular hemolysis and phagocytosis of infected RBCs. Intravascular hemolysis can eventually lead to hemoglobinuria and possibly renal failure if parasitemia is high. Potential laboratory findings in a malaria case

include normochromic/normocytic anemia, thrombocytopenia, leukopenia or leukocytosis, hyponatremia, hypoglycemia, increased liver and renal function tests, proteinuria, and abnormal coagulation tests.<sup>10</sup> The hematology results for the patient revealed anemia, thrombocytopenia, leukopenia, and elevated D-dimer. Chemistry and urinalysis demonstrated elevated transaminases, total and direct bilirubin, and protein and blood in the urine (Table 1). The thrombocytopenia and leukopenia may have been caused by splenomegaly and resultant sequestration. Thrombocytopenia may also be

accounted for by evidence of DIC in the patient, as suggested by the elevated D-dimer. Elevated total and direct bilirubin is a result of intravascular hemolysis.

Diagnosis of malaria is mainly based on the patient's clinical symptoms as well as findings during physical examination, although physical examination findings tend to be nonspecific. Evidence of hepatic dysfunction may be present. Malaria is also known to cause splenomegaly due to increased clearance of parasitized RBCs.<sup>10</sup> Laboratory testing must be performed for definitive diagnosis. Direct microscopic evaluation of thick and thin blood smears is the most common technique for identification. There are also malaria antigen tests (RDTs), and molecular tests for species differentiation or for use in those with low levels of parasitemia.<sup>6</sup> Evaluation of thick and thin Giemsa – stained peripheral blood smears, as mentioned previously, remains the gold standard and is an essential step in accurately identifying *Plasmodium* species. It is necessary to identify the species that causes an infection because it has an effect on the type of treatment protocol that is administered.<sup>3</sup> The Giemsa stain allows for the detection of morphological features that may be seen in specific species and lacking in others. In *P. ovale* infection, the RBCs that are infected are usually normal in size, but may be enlarged by 1.25X, as the organism infects young erythrocytes, which have more pliable membranes. This allows for distortion and enlargement of infected RBCs. The RBCs are round to oval in shape, may contain Schüffner's dots, and sometimes appear fimbriated. In the ring form trophozoite stage, the parasite has a solid cytoplasm and large chromatin. It is also common to see multiply-infected RBCs with *P. ovale*. Schizonts contain 6-14 merozoites. These are most of the morphologic characteristics that laboratory professionals follow in order to identify *P. ovale* as the cause of

malaria.<sup>11</sup>

Even though microscopic evaluation remains the gold standard for laboratory confirmation of malaria, sensitivity is not 100%, and the requirement of skilled handling and interpretation makes it difficult for clinical laboratory scientists practicing in non-endemic areas to maintain optimal proficiency.<sup>12</sup> Multiple laboratory tests have been developed over the years to aid in the rapid diagnosis of malarial infections.

Although a majority of the testing performed on the patient's specimen suggested malaria, it is important to investigate the BinaxNOW test kit in order to understand why it was not able to detect the malarial antigens present in the patient's blood. The BinaxNOW Malaria test (BN) is the first and only Food and Drug Administration (FDA) approved rapid test for malaria in the United States.<sup>13</sup> This qualitative test utilizes monoclonal antibodies to identify histidine-rich proteins that are specific for *P. falciparum*, and aldolase, which is a major enzyme in the glycolytic pathway of malarial parasites, in an EDTA whole blood specimen.<sup>1,6</sup> Commercial antibodies are separated out along a membrane; if malarial antigens are present when the specimen is added, they will bind to trapped antibodies on the membrane and form a visible test line under the control line after addition of reagent. This test does not have the ability to discriminate between non-*P. falciparum* species or infections with multiple *Plasmodium* species.<sup>1</sup> Test performance was assessed in a 2001 study that took place in malarial endemic regions. BinaxNOW was compared to traditional thick and thin peripheral blood smear evaluation. The diagnostic sensitivity was evaluated based on the levels of parasitemia observed microscopically. The study concluded that this immunochromatographic method was very useful for the diagnosis of *P. falciparum* cases, but not as effective for detection of other malaria species.<sup>1</sup>

Before analyzing the diagnostic sensitivity and specificity of the BinaxNOW test, it is essential to understand that to determine the sensitivity of the test, the number of parasites per  $\mu\text{L}$  of blood were used to express parasitemia levels rather than the percent parasitemia, which is what the laboratory uses when evaluating thin peripheral blood smears<sup>1</sup>. Parasitemia can also be quantified using the thick smear, but due to the ease of visibility of the organisms on the thin smear, it is most often used for

determination of the level of parasitemia. The percent parasitemia can be converted to the number of organisms per  $\mu\text{L}$ . Using a standard number of  $5.0 \times 10^6$  RBC per  $\mu\text{L}$ , a 1% parasitemia is equivalent to 50,000 organisms/ $\mu\text{L}$ . A 0.1% parasitemia is equivalent to 5,000 organisms/ $\mu\text{L}$ . The calculation can be adjusted if the exact number of RBCs per  $\mu\text{L}$  of blood is known for the patient.<sup>14</sup> It has been demonstrated that expert microscopy has the ability to detect a parasitemia of 0.001% (50 organisms per  $\mu\text{L}$ ).<sup>14</sup>

For *P. falciparum*, the results of the 2001 study conclude that with a parasitemia level of  $>5,000/\mu\text{L}$ , the diagnostic sensitivity of the test was 99.7%. The diagnostic sensitivity of the test dropped with lower levels of parasitemia. At a level of 0-100/ $\mu\text{L}$ , the diagnostic sensitivity was 53.9%. The overall diagnostic sensitivity was 95.3% for *P. falciparum* with a diagnostic specificity of 94.2%. This was a reliable percentage because the study involved 1,796 malaria cases that tested positive microscopically. Of the 1,796 patients, 557 of them were infected with *P. falciparum*, and 1,187 were due to *P. vivax* infections. For *P. vivax*, the diagnostic sensitivity at a parasitemia level of  $>5,000/\mu\text{L}$  was 93.5%. Sensitivity also dropped with lower levels of parasitemia. The overall diagnostic sensitivity was 68.9% for *P. vivax* with a diagnostic specificity of 99.8%. Mixed infections with *P. falciparum* and *P. vivax* accounted for 34 out of the 1,796 cases, with 94.1% sensitivity. The diagnostic sensitivity was lower for *P. malariae* and *P. ovale* cases. For *P. malariae*, the diagnostic sensitivity was only 43.8% for 16 microscopically positive malaria samples and 50% diagnostic sensitivity for two *P. ovale* samples. Therefore, the diagnostic sensitivity of the BinaxNow test kit was low for *P. ovale*, however, an insufficient number of positive samples for these parasites were tested in the study. For this reason, the diagnostic sensitivity and specificity for *P. ovale* cannot be accurately concluded.<sup>1</sup>

In a 2014 study by Tanizaki, Kato, Iwagami, et al, the authors concluded that the diagnostic sensitivity of RDTs such as the BinaxNOW test kit was not high enough to properly diagnose *P. ovale* infection. This conclusion was also based on a limited number of *P. ovale* samples, which resulted in only 22.2% sensitivity for the nine patients who were tested.<sup>15</sup> Furthermore, researchers from different institutions have discovered that the diagnostic sensitivity of RDTs for *P. ovale* in clinical samples can be as low as 5.5%.<sup>12</sup> It is known that patients

infected by *P. ovale* often have low parasitemia status.<sup>7</sup> Some studies also state that *P. ovale* might not be as easily detected using RDTs because this organism tends to have a low production of aldolase.<sup>16</sup> Therefore, clinical laboratories in the United States that utilize BinaxNOW as the primary screening test for malaria must be aware that even though it is an excellent test for *P. falciparum* and *P. vivax*, many *P. ovale* cases might be falsely negative, especially if direct microscopic examination of the peripheral blood smear is not performed at the same time. In our institution, all BinaxNOW tests that are ordered by physicians automatically get an order for a blood parasite smear as well. All patients regardless of the BinaxNOW result get a blood smear result with a parasitemia level. The final result is reported when the pathologist of hematology reviews the blood smear. The smear gets sent out to the CDC for confirmation when the pathologist is unable to differentiate between non-*P. falciparum* species (usually between *P. ovale* and *P. vivax*; it is rare that we have *P. malariae*). Almost all of the blood smears that have been sent out to the CDC in 2016 were confirmed to be *P. ovale* (one result is still pending). When a negative blood smear result occurs, the report to the health care provider reads, "No blood parasites seen. Although a negative smear renders the diagnosis of malaria unlikely, it is highly recommended that multiple specimens be examined over 2 to 3 days if clinical suspicion is high." The manufacturer of the BinaxNOW test kit states that all negative tests should be confirmed with microscopy.<sup>1</sup> Microscopy allows for speciation and determination of parasite load and disease severity. Malaria RDTs do not provide this information.<sup>12</sup>

Another notable fact is that the BinaxNOW malaria RDT does not have a high diagnostic sensitivity with *P. knowlesi* infection. In a 2014 study, 28 fresh blood specimens and 41 frozen specimens from patients with *P. knowlesi* were tested using the BinaxNOW malaria RDT. Speciation was done prior using PCR. The RDT was able to detect non-falciparum species in only 29% (8/28) in fresh blood specimens with *P. knowlesi*, and 24% (10/41) in frozen blood specimens.<sup>17</sup> *P. knowlesi* is a cause of human malaria in Southeast Asia. It is difficult to diagnose microscopically because the young ring-form trophozoites share morphologic characteristics with *P. falciparum* and the more mature trophozoites with *P. malariae*.<sup>18</sup>

It is also important to mention that high levels of

parasitemia can cause false negative results with RDTs due to the prozone effect. Excess antigen blocks binding sites on the test strip, thereby preventing the binding of antibodies. This can occur in specimens demonstrating a parasite load of 4% or greater.<sup>19</sup>

False positives may occur with RDTs because malarial antigens can remain in circulation for a few weeks after successful treatment.<sup>20</sup> Cross-reactivity with rheumatoid factor has been described. Rheumatoid factor can be present in autoimmune conditions, as well as in certain viral and parasitic infections.<sup>21</sup>

This case illustrates the importance of the clinical laboratory scientist to maintain competency, as detection, speciation, and estimate of parasitemia is still heavily reliant upon microscopic evaluation of peripheral blood smears. This is especially true with clinical laboratory scientists who are not practicing in malaria endemic areas. As stated previously, expert microscopy has been shown to have the ability to detect 50 organisms per/μL.<sup>14</sup> Clinical laboratory scientists who are new to the field and/or lack experience will most likely be unable to detect such a low level of parasitemia.

Even among expert microscopists there are discrepancies. In a 2012 study in which researchers were comparing RDTs with microscopy and PCR for detection of malaria in school-aged children in Kenya, well-trained “expert” technologists were used for the microscopic evaluation. During the evaluation of the peripheral blood smears, 10.2% (612) slides had to be re-stained due to poor staining, and 18.7% (1,125) had discrepancies between two expert microscopists, which had to be resolved by a third technologist.<sup>22</sup>

Poor technique (microscopy, preparation and staining of peripheral blood smears) can lead to inaccurate results. Artifacts such as erythrocytic inclusions, platelets, and stain precipitate can be mistaken for *Plasmodium* spp. Careful training and maintenance of skills in preparation and evaluation of peripheral blood smears is needed.<sup>23,24</sup> The authors recommend that clinical laboratory scientists regularly include malaria diagnostics as part of their continuing education.

## CASE CONCLUSION

After administration of artemether and lumefantrine, and primaquine phosphate, the patient began to show

signs of improvement, and the percent parasitemia eventually dropped to 0%. If Giemsa – stained thick and thin peripheral blood smears had not been performed in this case, the patient would not have been properly diagnosed, potentially leading to severe complications. A proper diagnosis was obtained because the physicians collected all of the pertinent patient history, including the patient’s signs, symptoms, and travel history. Also, the various laboratory tests that were ordered played a major role in aiding in the diagnosis of the patient because the hematology, microbiology, and chemistry tests correlated with the presentation of a malarial disease. This case portrays how essential it is for the clinical laboratory scientist to maintain competency in preparing and reading Giemsa – stained thick and thin peripheral blood smears in order not to overlook infections caused by *P. ovale*. In our institution in 2016, there were 5 confirmed cases of *P. ovale* that tested negative with the BinaxNOW RDT. These cases were identified by direct microscopic examination of the peripheral blood smears and confirmed by the CDC.

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