The Brief Case: A 6-Year-Old with Fever, Abdominal Pain, and Recent Travel to Sierra Leone

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CASE

A 6-year-old girl presented to the Nationwide Children’s Hospital emergency department in Columbus, OH, following a 3-day history of fever and 1 day of abdominal pain. She had recently returned from a 2-week trip to Sierra Leone and did not undergo any antimalarial prophylaxis. In the emergency department, she was found to be hypotensive and febrile and had altered mental status. Her maximum temperature from the day of admission was 40.1°C. She was started on broad-spectrum antibiotics, and laboratory testing was performed. Her initial laboratory results demonstrated a low hemoglobin of 8.9 g/liter, which dropped to 6.0 g/liter within 3 h (normal range, 11.5 to 15.5 g/liter), a total bilirubin elevated to 6.6 mg/dl (normal range, 0.1 to 1.0 mg/dl), and lactate dehydrogenase of 1,582 U/liter (normal range, 350 to 850 U/liter). These symptoms and lab findings prompted the clinical team to order a malaria evaluation, including thick and thin blood smears and an immunochromatographic malaria rapid diagnostic test (BinaxNOW Malaria; Abbott Laboratories, Abbott Park, IL). This assay detects the histidine-rich protein 2 (HRP2) antigen specific for Plasmodium falciparum on line T1, as well as a pan-malarial aldolase on line T2, which is expressed in all of the malaria species that infect humans: P. falciparum, P. vivax, P. ovale, and P. malariae (1). The screen for this patient generated T2 and control bands, indicating infection with P. vivax, P. ovale, or P. malariae (Fig. 1A). However, examination of peripheral blood smear showed morphologic findings consistent with P. falciparum species (frequent ring forms with more than one chromatin dot, multiple ring forms infecting the same red cell, and infected red cell size similar to noninfected red cells). Parasitemia was high, with more than 20% of red cells infected (Fig. 1B).

The patient was transferred to the intensive care unit and treated for severe P. falciparum malaria in collaboration with the CDC using intravenous artesunate followed by oral artemether-lumefantrine (2). She also developed severe acalculous cholecystitis, for which she received ciprofloxacin. Two units of red blood cells were administered on days 5 and 6 of her admission. Her smears were monitored, and by day 2, no parasites were detected: she was discharged 7 days after the initial presentation. Three weeks later, she had evidence of ongoing in vivo hemolysis: her hemoglobin remained low (9.5 g/liter; normal range, 11.5 to 15.5 g/liter), and she had elevated reticulocytes at 9.6% (normal range, 0.4 to 2.9%), haptoglobin of <30 mg/dl (normal range, 33 to 171 mg/dl), and lactate dehydrogenase of 1,167 U/liter (normal range, 350 to 850 U/liter). Her total bilirubin normalized at 0.8 mg/dl (normal range, 0.1 to 1.0 mg/dl). This is consistent with delayed hemolysis, which has been documented in malaria patients treated with artesunate (3).

DISCUSSION

This case demonstrates the prozone effect on malaria antigen detection as the result of hyperparasitemia with Plasmodium falciparum causing a false-negative result. The prozone effect occurs as the result of excessive amounts of either antigen or antibody that interferes
with the formation of the normal antigen-antibody complex required for signal generation in a diagnostic test. This is an uncommon phenomenon, as assays are usually designed to have excess capture and detection molecules, but can occur when the antigen is present in a very high concentration. As a result, the signal decreases with the increase in analyte concentration. This is sometimes referred to as the “hook effect.” In this case, hyperparasitemia with *P. falciparum* and associated HRP2 antigen overwhelmed the detection ability of the test. The prozone effect can be readily confirmed by diluting the patient sample. As the antigen concentration decreases by dilution, the signal increases. In the case of this patient, we diluted whole blood with 0.9% NaCl saline in a 1:1 dilution (Fig. 2A) and a 1:10 dilution (Fig. 2B), per World Health Organization (WHO) guidelines (4). As the dilutions increased, the band intensity increased, verifying a false negative from the prozone effect. Malaria-specific PCR confirmed that the infecting species was *P. falciparum*.

The prozone effect has been previously documented in malarial rapid diagnostic tests (RDTs) that detect HRP2 (4–7). It can lead to a negative signal (no visible test line) or weakened positive signal (faint or weak test line). In one study from Gillet et al., 17 RDTs that detect HRP2 were used to test 7 samples with *P. falciparum* hyperparasitemia (defined as >5% infected red cells). The samples were run neat and at 1:10, 1:50, and 1:100 dilutions with saline. All but one of these RDTs was affected by prozone (6). A

![Image](image-url)

**FIG 1** (A) BinaxNOW Malaria test results for the patient showing positive-control and T2 lines, indicating *P. vivax*, *P. ovale*, or *P. malariae* infection. (B) Blood smear with a high percentage of ring forms, including multiple ring forms in single cells (arrows) and normal-size red blood cells, which are consistent with *P. falciparum* infection.

![Image](image-url)

**FIG 2** The initial patient sample was diluted (A) 1:1 with saline and (B) 1:10 with saline. Only after dilution was a T1 line observable, indication of the prozone effect in a *P. falciparum* infection.
subsequent study attempted to establish the frequency of false-negative results from the prozone effect in a population where malaria is endemic; a total of 76 samples with hyperparasitemia of *P. falciparum* were tested on 10 RDTs that detect the HRP2 antigen. Six brands of RDTs demonstrated false-negative frequencies between 6.7 and 38.2% (5). In one RDT brand, 10.9% of samples with hyperparasitemia, 1.2% of all positive samples, and 0.1% of all samples tested in a 4-month period were affected by prozone. Of note, prozone is observed most frequently in children under age 5 years (5). The exact relationship between parasite density and prozone frequency is unclear. Early studies found prozone was seen in samples with both low and high levels of parasitemia (6). However, in a subsequent larger study, prozone occurred infrequently in samples with parasite densities of <0.4%, but more frequently in samples with higher density (5). In studies using a range of concentrations of recombinant HRP2 and cultured *P. falciparum* parasites, there was a clear relationship between increasing malarial protein or parasite concentration and decreasing line intensity in three RDTs (7). The prozone effect has only been demonstrated in tests that detect HRP2 antigen, and not in tests for *P. falciparum*-specific lactate dehydrogenase or pan-specific lactate dehydrogenase or aldolase; nevertheless, the choice of HRP2 over lactate dehydrogenase for use in *P. falciparum*-specific tests is based on its increased sensitivity and lower susceptibility to heat-induced damage (4, 5, 8).

Another important cause of false-negative RDTs is deletion of the *pfhrp2* and/or *pfhrp3* gene, which code for the HPR2 and HPR3 proteins, respectively (4, 9). The HPR3 protein can cross-react with HRP2-based RDTs (4). In cases of *pfhrp2*/*pfhrp3* deletion, the HRP2 protein is not made, and therefore is not detected by the RDT, causing a false negative. In 2010, a publication reported the first isolates of *P. falciparum* with deletion of the *pfhrp2*/*pfhrp3* genes from the Amazon region of Peru (9, 10). Since then, the *pfhrp2*/*pfhrp3* deletions have been identified at frequencies of ≥5% in cases from Colombia and Brazil, as well as in sub-Saharan Africa, particularly Eritrea. However, several other countries where malaria is endemic have also reported cases of *pfhrp2*/*pfhrp3* deletions, though the prevalence has remained low (4). In Peru, one study of 148 samples identified 41% with deletion of *pfhrp2* and over 20% with deletions of both *pfhrp2* and *pfhrp3* (9). In cases where *pfhrp2*/*pfhrp3* deletions are suspected, diagnosis of *P. falciparum* infection and confirmation of the deletion can be made by PCR. The WHO recommends that in locations with ≥5% of malaria cases with *pfhrp2*/*pfhrp3* deletions, RDTs be switched from HRP2 detection methods to those that detect *P. falciparum* by another antigen, such as *Plasmodium* lactate dehydrogenase, although these are generally not as sensitive as HRP2 RDTs. Moreover, the WHO encourages that where microscopy is available, there should be increased education in parasitological confirmation of RDTs (4). In the case reported here, it was unlikely that this patient was infected with *P. falciparum* with a *pfhrp2*/*pfhrp3* deletion, as to date, no parasites with these deletions have been identified in Sierra Leone, where the patient had just visited. False negatives from prozone can be distinguished from *pfhrp2*/*pfhrp3* deletions, as deletions do not become positive for *P. falciparum* upon dilution.

Other causes of false-negative results in malaria RDTs include test cassette failure, user error or misinterpretation, and parasite density below the limit of detection. Generally, RDTs have very good sensitivity for *P. falciparum* with only 0.002% of red cells infected. False negatives from low parasite densities can be addressed by repeat testing at a later time (6).

False-positive reactions can also occur, although reported with lower frequency. They have been documented in patients with high concentrations of rheumatoid factor (6). Additionally, false-positive results may be seen in patients with chronic infections, often seen in areas where malaria is endemic, and with persistence of HRP2 in circulation following parasite clearance.

This case illustrates the importance of recognizing false-negative *P. falciparum* results when using RDTs and the need to evaluate negative RDTs for the prozone effect by dilution or by determining the presence of *pfhrp2*/*pfhrp3* deletions by PCR in symptomatic patients. It also highlights that blood smears, though not easily performed in all settings, are still considered the “gold standard” for malaria diagnosis and should be done in conjunction with RDTs when possible. In a clinical lab with the available resources, screen results should be available when the blood smears are read. If *P. falciparum* is identified
microscopically, but not by the RDT, causes of false-negative identification of *P. falciparum* should be considered. Otherwise, diagnosis may be delayed or missed, especially if the RDTs used do not include a pan-malaria antigen.

**SELF-ASSESSMENT QUESTIONS**

1. What is not a possible cause of a false-negative result for *P. falciparum* on a malaria RDT?
   a. Low parasite density
   b. High concentrations of rheumatoid factor
   c. Deletion of *pfhrp2/3* genes
   d. Prozone effect

2. The prozone effect can occur as the result of which of the following?
   a. Excess detection and capture molecules in the assay
   b. Excess antigen present in the patient sample
   c. Misinterpretation of the assay results
   d. Use of tests other than HRP2 for the detection of *P. falciparum*

3. What additional testing can be done if a false-negative HRP2 result due to *pfhrp2/3* deletions is suspected in a particular patient?
   a. PCR to confirm *P. falciparum* infection
   b. PCR to identify *pfhrp2* and/or *pfhrp3* deletion
   c. Switch to an RDT that uses a non-HRP2 method of detection
   d. All of the above

**REFERENCES**