SHORT REPORT: RECOVERY AND USE OF *PLASMODIUM* DNA FROM MALARIA RAPID DIAGNOSTIC TESTS

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Abstract. The purpose of this study was to show that individual malaria rapid diagnosis tests (MRDTs) could also be used to isolate *Plasmodium* DNA for genetic studies. We extracted and amplified *Plasmodium* DNA using two commercial MRDT kits. Phenol/chloroform extraction followed by a nested polymerase chain reaction (PCR) can be used to identify *Plasmodium falciparum* and *Plasmodium vivax* from MRDTs. The PCR on MRDT-isolated DNA was more sensitive than antigen capture by MRDT. Satisfactory results were also obtained if older MRDT tests were used, even after long periods of storage at ambient temperature, with no special preservation.

The recovery of *Plasmodium* DNA from routine Giemsa-stained blood smears has been previously described. DNA extraction from blood smears is technically feasible and may be used to confirm a diagnosis or for retrospective and prospective molecular studies. However, there are risks of contamination during staining, microscopic examination, and storage of the smears. Malaria rapid diagnosis tests (MRDTs) are very useful in isolated places with no available microscope and the risk of contamination between tests is minimal. The purpose of this study was to demonstrate that MRDTs conducted for individual diagnosis could also be used, without particular preservation, to conserve *Plasmodium* DNA for later genetic studies.

*Plasmodium* DNA was extracted and amplified using two commercial MRDT kits: Optimal® (DiaMed AG, Cressier sur Morat, Switzerland) and ICT Now® (Binx Inc., Portland, ME). These immunochromatographic tests are based on blood hemolysis and the subsequent reaction between monoclonal antibodies and antigens such as parasite lactate dehydrogenase (Optimal®) and histidine-rich protein II (ICT Now®). Capillary forces produced by blotting paper located at the top of a nitrocellulose membrane capture the blood sample. After migration on this membrane, colloidal gold red bands appear if the sample is positive.

We first tested the recovery of *Plasmodium* DNA. We extracted DNA from MRDT blotting paper. We tested three different extraction methods: the saponin/Chelex 100 method, the QIAamp DNA mini blood kit (Qiagen, Valencia, CA), and the phenol/chloroform method. For each extraction method, we used 10 MRDT positive samples with the same malaria-positive blood solution (50,000 *Plasmodium falciparum*/µL). According to the manufacturer’s instructions, 10–20 µL of blood should be used for the MRDT. The part of the blotting paper impregnated with blood was cut into small pieces (3 mm × 1 mm) and immersed in 600 µL of TNE buffer (0.15 M NaCl, 10 mM Tris HCl, 1 mM EDTA, pH 8) supplemented with 0.5% Triton X-100, 0.5% sodium dodecyl sulfate, and 5 mg/mL of proteinase K. Samples were incubated for one hour at 37°C, tubes were periodically shaken and centrifugated (4,000 × g for 30 seconds) at ambient temperature.

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DNA was extracted from the supernatant and precipitated at -20°C in absolute ethanol containing 400 mM sodium acetate, pH 5.2. The nested polymerase chain reaction (PCR) used to identify *Plasmodium* species has been previously described; amplifications were performed in duplicate. We determined the size of the amplicons by agarose gel electrophoresis in the presence of 0.5 µg/mL of ethidium bromide. We found that the phenol/chloroform method was the most effective.

Five negative MRDT (three Optimal® and two ICT Now®) and 35 positive MRDT (25 Optimal® and 10 ICT Now®) samples, all initially used for routine individual diagnosis, were used. All patients were living in French Guiana and the MRDTs were obtained from various health centers in French Guiana. Microscopy was carried out to confirm the diagnosis of malaria in all positive cases (23 were positive for *P. falciparum*, 12 for *P. vivax*, and 5 were negative). The corresponding infected patients had parasite densities of 400–100,000 *Plasmodium*/µL.

We compared the sensitivity thresholds of MRDTs and PCR on MRDT-isolated DNA by serially diluting blood specimens from two patients, one infected with *P. falciparum* and another infected with *P. vivax*, in blood from a malaria-negative subject of the same ABO blood group. Seven dilutions, ranging from 50,000 to 1.2 parasites/µL of blood, were used for *P. falciparum* and *P. vivax*. Microscopy was used as a control for each dilution. The PCR on blood and on DNA isolated with the MRDT was performed in duplicate.

We investigated the effect of storage duration by also investigating 10 old MRDTs: two Optimal® MRDTs used six months earlier, three Optimal® MRDTs, and four ICT Now® MRDTs used 12 months earlier, and one ICT Now®MRDT used three years earlier. All 10 MRDTs had been stored in ambient conditions (temperature = 25–30°C, humidity = 70–85%).

The phenol/chloroform method gave the highest extraction yields in malaria-positive PCR tests for all (10 of 10) MRDT (Optimal) tests conducted with the same malaria-positive blood solution. With the Chelex and Qiagen extraction methods, only 4 of 10 and 2 of 10 tests, respectively, were positive. We therefore used the phenol chloroform method for all subsequent extractions.

The results obtained with MRDT initially used for routine individual diagnosis and for PCR on MRDT-extracted DNA are shown in Figure 1. Four of the five negative MRDT samples did not yield an amplicon for *P. falciparum* or *P.
Results obtained with the Optimal® malaria rapid diagnostic test (MRDT) and polymerase chain reaction PCR on DNA extracted using the Optimal® MRDT. PV = Plasmodium vivax; Neg. = negative; Pf = P. falciparum. In cases of a positive reaction for P. falciparum in the Optimal® MRDT, the possibility of a mixed infection with P. vivax, P. ovale, or P. malariae cannot be excluded (see case 4, which was a mixed infection of P. falciparum and P. vivax). Case 6 was negative with the Optimal® MRDT but positive for P. falciparum and P. vivax when DNA isolated with the Optimal® MRDT was used. This figure appears in color at www.ajtmh.org.

vivax. The fifth showed positive amplification for P. falciparum and P. vivax (patient 6, Figure 1). Microscopy-based diagnosis was performed for all of these patients in peripheral health centers, where it was only possible to detect cases in which parasitemia levels exceeded 50–100 Plasmodium/μL. Twenty-three patients tested positive by both the Optimal® MRDT and PCR: 14 tested positive for P. falciparum, 8 for P. vivax, and one Optimal® MRDT was positive for P. falciparum, whereas the corresponding PCR amplification was positive for P. falciparum and P. vivax (patient 4, Figure 1). However, if a positive reaction for P. falciparum is obtained in the Optimal® MRDT, the possibility of a mixed infection with P. vivax, P. ovale, or P. malariae cannot be excluded.9 Two samples (one for P. falciparum and one for P. vivax) positive with the Optimal® MRDT showed negative results with the PCR. In these two cases, the person carrying out the test may not have followed the Optimal® MRDT protocol correctly. The blood on the blotting paper in these cases was much paler than would normally be expected (pale red rather than dark red).

We carried out PCR tests on DNA from 10 positive ICT Now® MRDT samples. The seven that were positive for P. falciparum were also positive for P. falciparum by PCR amplification. The three that were positive for P. vivax were also positive by P. vivax PCR amplification.

We compared the sensitivities of microscopy, the Optimal® MRDT, PCR on Optimal® MRDT-isolated DNA, and PCR on whole blood DNA (Table 1). Microscopy detected a minimum parasitemia level of approximately 5 parasites/μL of blood in our assays, whereas the examination result was considered negative only after analysis of a thick blood smear sample including at least 1,200 leukocytes (i.e., ~0.20 μL). The threshold for positive results was the same for P. falciparum and P. vivax amplifications. The Optimal® MRDT also showed no difference in the sensitivity thresholds for P. falciparum and P. vivax. The PCR on Optimal® MRDT-isolated DNA appeared to be 10 times more sensitive than Optimal® MRDT itself. This low sensitivity of the MRDT may account for the positive PCR results obtained with certain negative Optimal® MRDT results. The amplification of DNA from 15 μL of whole blood could be used to detect parasitemia levels as low as 3.7 × 10⁻⁵% (approximately 1.2 parasites/μL of blood in our assays). The sensitivities recorded here were similar to those reported in a previous study using the same amplification protocol and DNA extracted from whole blood.7 The detection threshold of the PCR on blood was 40 times higher than that of the control PCR on Optimal® MRDT-isolated DNA. Before migration on the nitrocellulose membrane, blood is lysed and diluted and the blotting paper

### Table 1

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<th>Parasites/μL of blood</th>
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<td>Microscopic examination for Pv</td>
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<td>Optimal® with Pv</td>
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<td>PCR for Pf from Optimal® with Pf</td>
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* Blood specimens were obtained from two patients, one infected with P. falciparum (Pf) and another infected with P. vivax (Pv), were serially diluted in blood from a malaria-negative subject from the same ABO blood group. Seven dilutions from 50,000 to 1.2 parasites/μL of blood were performed for P. falciparum and P. vivax. MRDT = malaria rapid diagnostic test; PCR = polymerase chain reaction.

† PCR was performed with approximately the same amount of blood used for MRDT (15 μL).
does not capture the entire volume. Moreover, molecular examination of membranes confirmed that some parasite DNA remained on the membrane at the end of the test. This may account for the observed loss of sensitivity. Lysis and dilution buffer incubated with 10 μL of parasitized blood (50,000 *P. falciparum* /μL) were collected and used for amplification. Solutions were tested separately and together, with and without migration on the MRDT. No PCR inhibitors were found in the lysis or dilution buffers used for the Optimal® and ICT® MRDTs.

The MRDT can be stored in ambient conditions with no risk of contamination. Each test is stored in a plastic (Optimal® MRDT) or cardboard (ICT Now® MRDT) box, which is closed after use, making it possible to store the test safely for long periods. However, the amplification of *Plasmodium* DNA from fresh blood was 100 times more sensitive than amplification from dried blood on blotting paper. We investigated the impact of storage duration on DNA extraction yield. All amplifications of DNA from positive Optimal® MRDT samples and positive ICT Now® MRDT samples stored for 6–36 months before use were positive (10 kits tested). These results are consistent with those of a previous study showing that storage for four years does not affect DNA quality. Overall, our results suggest that positive MRDT (such as Optimal® and ICT Now®) samples are a good source of malaria parasite DNA for retrospective and prospective genetic studies without additional population sampling.

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