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Comparison of IsoCode STIX and FTA Gene Guard Collection Matrices as Whole-Blood Storage and Processing Devices for Diagnosis of Malaria by PCR

KATHLEEN J. Y. ZHONG,1 CAROLA J. SALAS,2 ROBYN SHAFER,1 ALEX GUBANOV,1 ROBERT A. GASSER, JR.,3 ALAN J. MAGILL,2 J. RUSS FORNEY,4 AND KEVIN C. KAIN1

Centre for Travel and Tropical Medicine, Division of Infectious Diseases, Department of Medicine, Toronto General Hospital and the University of Toronto1; Department of Parasitology, U.S. Naval Medical Research Center Detachment, Lima, Peru2; and Department of Immunology3 and Department of Parasitology,4 Walter Reed Army Institute of Research, Silver Spring, Maryland

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We compared two collection devices, IsoCode and FTA, with whole blood for the diagnosis of malaria by PCR (n = 100). Using whole blood as the reference standard, both devices were sensitive for the detection of single-species malaria infections by PCR (≥96%). However, the detection of mixed infections was suboptimal (IsoCode was 42% sensitive, and FTA was 63% sensitive).

The isolation of high-quality template DNA from blood samples is important for diagnostic and molecular studies of malaria. Whole blood has frequently been used as the source of template DNA; however, collecting and transporting blood samples, especially from field sites, is problematic (1, 6, 8, 10, 11). Several filter paper devices have recently been marketed for the collection, storage, and processing of whole-blood samples for PCR. There have been no direct comparisons of the performance characteristics of these products. Differences in DNA template quality and subsequent PCR amplification among these different collection devices could have a considerable impact on outcome measures in malaria field trials.

In this study, we evaluated two commercially available blood collection devices, IsoCode STIX (Schleicher & Schuell, Keene, N.H.) and FTA Gene Guard card (Gibco BRL, Rockville, Md.), against whole blood for the detection and species identification of malaria parasites by PCR.

Blood samples were collected from individuals presenting with headache and fever (≥38°C) to an outpatient malaria clinic in Iquitos, Peru. Thick and thin blood smears were prepared and examined by experienced microscopists (≥200 fields of a thick smear; 1,000× oil immersion). Patients who were smear positive for malaria and who provided informed consent were eligible for inclusion in the study. Whole-blood samples (pretreatment) obtained from consecutive smear-positive malaria-infected patients via venipuncture in EDTA anticoagulant were collected on IsoCode STIX and FTA Gene Guard card collection devices according to the manufacturers’ directions. An aliquot of each blood sample was frozen and stored at −70°C. The IsoCode STIX and FTA filter paper samples were air dried and stored in individual plastic bags at room temperature until they were processed. DNA was extracted from the blood samples collected by the three methods.

* Corresponding author. Mailing address: Centre for Travel and Tropical Medicine, Division of Infectious Diseases, Toronto General Hospital, 200 Elizabeth St. MSG 2C4 Toronto, Canada. Phone: (416) 340-3535. Fax: (416) 595-5826.

Genomic DNA was extracted from the frozen whole-blood samples with a QIAamp blood extraction kit (Qiagen, Chatsworth, Calif.). Genomic DNA was extracted from the IsoCode and FTA devices according to the manufacturers’ directions. Each of the 300 DNA extracts was subjected to the same amplification method for a fragment of the plasmodial small-subunit RNA gene as described previously (10). Based on previously established PCR methodology using whole-blood samples, an amount of DNA extract equivalent to 5 μl of the original whole-blood specimen was used (6, 8, 11). In order to improve sensitivity and make the methods comparable, we modified the IsoCode manufacturer’s recommendations and used 25 μl of extract (5 μl of the original sample) in amplification reactions. Similarly, an FTA blood dot sample equal to 5 μl of the original whole-blood sample was used. The PCR products were electrophoresed and analyzed on 2% agarose gels. An independent observer, unaware of the DNA collection and extraction method used and the results of microscopy, interpreted the results of each amplification reaction. Positive and negative controls were included in each amplification assay. This study was approved by the Institutional Review Board of the U.S. Department of Defense and the Peruvian Ministry of Health.

Blood samples from 100 consecutive malaria-infected patients from Peru were included. We utilized a nested-PCR-based method using DNA extracted from frozen whole-blood samples as the reference standard on the basis of its previously demonstrated performance characteristics (1, 8, 10).

The DNA extraction procedures for IsoCode and FTA samples were simple to perform. Most users reported that the IsoCode strips were easier to use and manipulate. The time required for DNA extraction from frozen whole-blood on Qia-gen columns was 20 min; for IsoCode, the time was 35 min, and for FTA, it was 85 min.

The amplification results are presented in Table 1. Although both devices were sensitive for the detection of single-species infections, the use of IsoCode devices allowed the detection of only 8 of 19 mixed infections (42%; P = 0.003; Yate’s corrected chi square test) and FTA detected only 12 of 19 mixed infec-
TABLE 1. PCR results with whole blood versus IsoCode STIX and FTA Gene Guard card for 100 consecutive malaria samples

<table>
<thead>
<tr>
<th>Infecting species</th>
<th>PCR with whole blood</th>
<th>PCR with IsoCode STIX</th>
<th>% Sensitivity (95% CI)</th>
<th>PCR with FTA Gene Guard card</th>
<th>% Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>33 Pf</td>
<td>33 Pf</td>
<td>100 (96.6–100)</td>
<td>32 Pf</td>
<td>97.0 (91.2–100)</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>48 Pv</td>
<td>46 Pv</td>
<td>95.8 (90.1–100)</td>
<td>48 Pv</td>
<td>100.0 (97.2–100)</td>
</tr>
<tr>
<td>Mixed <em>P. falciparum</em> and <em>P. vivax</em></td>
<td>19 Pf and Pv</td>
<td>8 Pf and Pv</td>
<td>42.1 (19.9–64.3)</td>
<td>12 Pf and Pv</td>
<td>63.2 (41.5–84.9)</td>
</tr>
<tr>
<td>Total (n)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* a Malaria infections are classified by PCR results from whole-blood samples. No *Plasmodium ovale* or *Plasmodium malariae* infections were identified by microscopy or by PCR.

b Pf, *P. falciparum*; Pv, *P. vivax*; Neg, negative; CI, confidence interval.

...requirements to improve the detection of malaria. Both devices displayed good sensitivity for the subsequent PCR identification of single-species infections. However, neither was sensitive for the detection of mixed infections. The reasons for decreased detection of mixed infections are unknown but may reflect loss of template DNA through degradation or trapping of parasite DNA in the paper matrix (1). Since PCR-based assays are increasingly being used as reference standards for malaria diagnostic and treatment studies, these limitations may be important, since they may lead to errors in treatment and misclassification of outcomes.

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