Performance of a High Sensitivity Muse® Malaria P.f.-P.v. Detection Assay in a Study in Lagos, Nigeria

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Abstract

Effective malaria diagnosis is critical in the implementation of current malaria case management strategies. The need for the development of highly-sensitive detection methods that can also provide specification of malarial parasites has been amplified. While microscopy is considered the gold standard, it requires a high degree of expertise and training to provide reliable and sensitive results. A new method has recently emerged, which uses microcapillary cytometry on the low-cost Guava® Muse® Cell Analyzer along with the Muse® Malaria P.f.-P.v. Detection Assay. The assay uses multiplexing to confirm the presence of malarial parasite antigens by detecting HRP2, LDH, and pLDH antigens, with high-detection sensitivity as low as a few parasites/µL.

In this study, we evaluated the use only Muse Malaria P.f.-P.v. Detection Assay with fresh EDTA-whole blood samples in our laboratory in Lagos, Nigeria. In total, 62 adult and pediatric samples with parasitemia of 19-450,000 parasites/µL were examined. Data was obtained using three methods: malaria microscopy of Giemsa-stained blood films; the SD Bioline P.f./Pan Malaria Rapid Diagnostic Test (RDT) (available from Abbott and the Muse® Malaria Detection Assay. The results from the Muse assay demonstrated excellent agreement when compared to both expert microscopy and RDTs. Clear shifts were observed from low to high parasitemia, making interpretation simple. Compared to expert microscopy, the sensitivity of the Muse Malaria assay was 100%, and the specificity was 95.24%. It was noted that while Muse HRP2 and RDT HRP2 test results were similar to microscopy, pLDH RDT results were much less sensitive compared to microscopy. Muse P.f.-LDH results showed correspondence to microscopy results. The assay with dual high-sensitivity P. falciparum and P. vivax detection, with high-confidence when analyzing P. falciparum samples, and ensured that P. vivax-LDH antigens were not being missed. The Muse Malaria P.f.-P.v. Detection Assay can be a valuable, high-sensitivity tool in research laboratories to ensure Plasmodium antigens are appropriately detected and specified.

Materials and Methods

Malaria Study in Lagos, Nigeria

Figure 1. The Muse® Malaria P.f.-P.v. Detection Assay is a bead-based assay for malaria identification and Plasmodium typing on the Guava® Muse® Cell Analyzer. The assay detects Plasmodium vivax-LDH, P. falciparum-LDH, and P. falciparum-HRP2; negative samples show no shift of beads, while positive P. vivax and/or P. falciparum samples show shifts of the respective bead.

Figure 2. Whole blood from 62 adult and pediatric patients was collected in Lagos, Nigeria and analyzed at the ANDI Centre of Excellence for Malaria Diagnosis. The blood samples were examined for the detection and speciation of Plasmodium infections utilizing Giemsa-stained blood films, SD Bioline P.f./Pan RDT, and the Muse® Malaria P.f.-P.v. Detection Assay. In this study, a developmental version of software on a laptop attached to the Guava® Muse® System was used. Samples were analyzed within 36 hours of collection.

Figure 3. Example of results from fresh whole blood samples collected in Lagos, Nigeria. The utility of the Muse® Malaria P.f.-P.v. Detection Assay to identify and provide results across both adult and pediatric sample types are shown (A). A wide range of sample types were analyzed in the study, with parasitemia levels ranging from 19 to 459,089 parasites/µL (B). The Muse Malaria P.f.-P.v. Detection Assay provided the strong and clear indication of P.f.-HRP2 and P.f.-LDH across all sample ranges studied.

Results

Performance of Muse® Malaria P.f.-P.v. Detection Assay

Sensitivity and Specificity of the Muse® Malaria P.f.-P.v. Detection Assay

Table 1: Sensitivity and Specificity of the Muse® Malaria P.f.-P.v. Detection Assay

<table>
<thead>
<tr>
<th>Assay Output</th>
<th>HRP2</th>
<th>LDH</th>
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<tbody>
<tr>
<td>Microscopy</td>
<td>100%</td>
<td>78.05%</td>
</tr>
<tr>
<td>RDT HRP2 vs. Microscopy</td>
<td>95.24%</td>
<td>95.24%</td>
</tr>
<tr>
<td>RDT µLDH vs. Microscopy</td>
<td>95.46%</td>
<td>95.46%</td>
</tr>
</tbody>
</table>

Conclusions

• The Guava® Muse® Cell Analyzer, along with the Muse® Malaria P.f.-P.v. Detection Assay, use multiplexing to confirm the presence of malarial parasite antigens in human whole blood by detecting HRP2, LDH, and pLDH antigens in parallel.
• In this study, 62 fresh whole blood samples were evaluated using the Muse Malaria P.f.-P.v. Detection Assay, microscopy of Giemsa-stained blood films, and a commercially available malaria RDT. Parasitemia levels of samples ranged from 19-450,000 parasites/µL as determined by microscopy.
• The Muse Malaria P.f.-P.v. Detection Assay provided results that were highly comparable to microscopy results. In addition, both P.f.-HP2 and P.f.-LDH antigens demonstrated results comparable to microscopy. The Muse P.f.-LDH Detection Assay showed superior sensitivity compared to RDT’s for the detection of the LDH antigen.

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