Drug resistance to sulfadoxine-pyrimethamine (SP) is widespread in Malawi and is conferred by mutations in dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). The combination of five mutations (DHFR-51, -59, and -108, and DHPS-437 and -540) has been strongly associated with SP failure. Other mutations, such as DHFR-164, DHPS-581, and DHPS-613, develop later and sequentially add to SP resistance. In addition, mutations in malaria have been previously described, and the successful probe had the following relative to the single nucleotide polymorphism using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, LaJolle, CA). An annealing reaction consisting of 8 µL of PCR product (either a control or sample DNA) was mixed with 1 µL of 10× annealing buffer (1 M NaCl, 100 mM Tris-HCl, pH 7.5, 20 mM EDTA), 2 µL of 6× loading dye, 0.25 µL of 100 pM forward primer, 0.25 µL of 100 pM reverse primer, and 0.5 µL of radiolabeled probe in a total volume of 12 µL. All HTA gels included the following controls: water, a non-template control PCR, and PCRs from wild-type (MR4, MRA-102G) and mutant (MR4, MRA-176G) DNA stocks. Gels were analyzed as described previously.

Briefly, wild-type DHFR is amplified at the region of interest. The PCR was carried out in a 50-µL reaction containing 5 µL of DNA, 1.25 units of HotStar Taq DNA polymerase (Qiagen), 5 µL of 10× PCR buffer, 1 µL of dNTP mixture (catalog no. U1511; Promega, Madison, WI), 300 nm forward primer (5′-ATCATTAACAAAGTTGAGACATCTAATAGTTTAC-3′), and 500 nM reverse primer (5′-TCCGTAACGAAAT-AATTGTGATCTCAT-3′). This reaction was amplified by preheating to 95°C for 15 minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute. The reaction was completed with a 10-minute hold at 72°C.

DNA was extracted from filter paper blood spots or frozen blood using the Qiamp DNA mini kit (Qiagen, Valencia, CA). The method for developing HTAs for the detection of point mutations in malaria has been previously described, and the differences for the DHFR-164 HTA are noted below.

<table>
<thead>
<tr>
<th>% Mutant control DNA</th>
<th>% Mutant detected (replicate 1)</th>
<th>% Mutant detected (replicate 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>47.9</td>
<td>42.1</td>
</tr>
<tr>
<td>20</td>
<td>22.3</td>
<td>16.3</td>
</tr>
<tr>
<td>10</td>
<td>9.3</td>
<td>11.1</td>
</tr>
<tr>
<td>5</td>
<td>5.9</td>
<td>6.9</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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From both samples, a selection of 3 mutant and 2 cloned per protocol (Invitrogen). Twenty-five colonies from type parasites. The DHFR-164 mutation is present only in patients with wild-type at all three DHFR sites.

The presence of this mutation in only one of the two cohorts studied here could serve as an important lesson. The samples from Mpemba and Madziabango were taken early in pregnancy, before subjects received SP IPTp. The samples from Queen Elizabeth Central Hospital were obtained at labor and delivery, after the women had probably received IPTp. This suggests that the difference might be in part caused by in vivo selection for the DHFR-164L genotype. Thus, monitoring for the DHFR-164 mutation should be continued. The ability of the HTA to detect DHFR-164L-bearing variants representing ≥ 1% of the parasites in an individual host suggest that the method is more sensitive than standard PCR methods and should be used if proof of the mutation’s absence is needed.

Thus, this report confirms the presence of the DHFR-164L mutation in Malawi by a second assay method as well as DNA sequencing. We also demonstrate 100% concordance for the presence of mutant DHFR-164 between the HTA and real-time PCR assay.

The DHFR-164 mutation remains rare in Africa, but has been documented in Central African Republic, Kenya, Uganda, the Comoros, Tanzania, and among travelers returning to Sweden from Africa. These observations, together with the data presented here, suggest that DHFR-164L is a problem in Africa. Thus, monitoring for the DHFR-164 mutation should be continued. The ability of the HTA to detect DHFR-164L-bearing variants representing ≥ 1% of the parasites in an individual host suggest that the method is more sensitive than standard PCR methods and should be used if proof of the mutation’s absence is needed.

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REFERENCES


