The Search for *Plasmodium falciparum* histidine–rich protein 2/3 Deletions in Zambia and Implications for *Plasmodium falciparum* histidine–rich protein 2-Based Rapid Diagnostic Tests

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Abstract. We attempted to identify *Plasmodium falciparum* histidine–rich protein 2 (pfhrp2/3) deletions among rapid diagnostic test (RDT)–negative but PCR- or microscopy-positive *P. falciparum*–infected individuals in areas of low transmission (Choma District, 2009–2011) and high transmission (Nchelenge District, 2015–2017) in Zambia. Through community-based surveys, 5,167 participants were screened at 1,147 households by *P. falciparum* histidine-rich protein 2 (PFHRP2)-based RDTs. Slides were made and dried blood spots were obtained for molecular analysis. Of 28 samples with detectable *P. falciparum* DNA, none from Nchelenge District were pfhrp2/3 negative. All eight samples from Choma District had detectable pfhrp3 genes, but pfhrp2 was undetectable in three. DNA concentrations of pfhrp2-negative samples were low (< 0.001 ng/µl). These findings suggest that PFHRP2-based RDTs remain effective tools for malaria diagnosis in Nchelenge District, but further study is warranted to understand the potential for pfhrp2/3 deletions in southern Zambia where malaria transmission declined over the past decade.

The availability of *Plasmodium falciparum* histidine-rich protein (PFHRP) 2–based rapid diagnostic tests (RDTs) has dramatically improved parasitological confirmation of suspected malaria cases in resource–limited settings. Recently, numerous studies reported *P. falciparum* parasites lacking pfhrp2 and pfhrp3 genes in Africa, including the Democratic Republic of the Congo, Eritrea, Ghana, Kenya, Mali, Mozambique, Rwanda, and Senegal.¹⁻³ The pfhrp3 gene is highly homologous to pfhrp2,⁴ and parasites lacking both pfhrp2 and pfhrp3 genes, or substantial parts of these genes, do not express functional proteins and are not detected by PFHRP2-based RDTs.¹

In Zambia, the National Malaria Control Center introduced PFHRP2-based RDTs in 2005 and achieved national-level scale-up in 2009.⁵ Zambia has made significant progress in reducing malaria transmission and aims to eliminate malaria by 2021. To achieve this goal, the use of PFHRP2-based RDTs has expanded beyond the diagnosis of suspected cases to also be used to screen individuals residing in proximity to symptomatic index cases through reactive case detection.⁶ Despite the expanded use of PFHRP2-based RDTs, the extent of pfhrp2/3 deletions in Zambia is unknown. We attempted to identify pfhrp2/3 deletions using blood samples collected during community-based, active case detection in Choma and Nchelenge districts, Zambia.

Choma District in the Southern Province has seasonal malaria transmission, and PFHRP2-based RDTs were introduced in 2007.⁷ As malaria transmission has declined, Choma District is considered a pre-elimination setting (malaria prevalence by RDT < 1%).⁸,⁹ By contrast, malaria transmission in Nchelenge District, Luapula Province, is high with little seasonal fluctuation. Malaria control interventions have been only modestly effective and malaria prevalence by RDT is approximately 50%.⁹,¹⁰ Luapula Province is one of the four focus provinces where the National Malaria Elimination Program supports additional malaria control efforts, including school-based bed net distribution, expansion of community case management, and training of health providers in the management of severe malaria.⁶

Random sampling based on satellite imagery was used to select households for participation in community-based surveys in between 2009 and 2011 in Choma District, and between 2015 and 2017 in Nchelenge District. Briefly, satellite images of the catchment area were used to create a sampling frame from which households were randomly selected for participation in community-based, serial cross-sectional surveys. Trained local field-workers used global positioning system coordinates to locate selected households for initial notification visits and data collection. All household residents older than the age of 3 months present at the time of the visit were eligible for enrollment, and written informed consent was obtained from all adults or caregivers of children who agreed to participate. Tympanic temperature was taken, and participants were tested for malaria by a PFHRP2-based RDT (Choma District: ICT Malaria *P. falciparum* ICT Diagnostics, Cape Town, South Africa); Nchelenge District: SD Bioline Malaria Ag *P. falciparum* [Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea]). Different RDTs were deployed at the two sites based on a change in the Zambian Ministry of Health guidelines in 2013. Both RDTs met World Health Organization procurement criteria and reliably detect parasite densities of 200 parasites/µL or higher.¹¹,¹² Using finger-prick blood, dried blood spots (DBS) were prepared for molecular analysis. All RDT-negative participants were offered treatment according to the guidelines of the Zambian Ministry of Health.

In Choma District, 2,183 residents from 414 households agreed to participate in the study. In Nchelenge District, 2,984
residents from 733 households agreed to participate. Samples were transported to laboratories at Macha Research Trust in Choma District or the Tropical Disease Research Centre in Ndola. DNA was extracted from the DBS and screened using a nested-PCR targeting the mitochondrial cytochrome b gene (cytb), which is conserved among the major human *Plasmodium* species. To exclude non-*P. falciparum* malaria infection, which results in a negative PFHRP2-based RDT, all malaria infection, which results in a negative *P. falciparum* infection, were further screened by qPCR for *P. falciparum* DNA, the detection limit of the pfhrp2 and pfhrp3 assays. Samples with less than 0.0001 ng/μL parasite-specific DNA, the detection limit of the pfldh qPCR and the pfhrp2/3 PCR, were considered to have insufficient parasite DNA and were excluded from further analysis. Of those samples with sufficient DNA (Choma District = 8, Nchelenge District = 28), three samples from Choma District failed to amplify pfhrp2 (Table 1). All three samples with undetectable pfhrp2 were pfhrp3 PCR positive. Among these three samples with undetectable pfhrp2 but detectable pfhrp3 from Choma District, the presence of parasite DNA was confirmed using PCR targeting pfcr. None of the RDT-negative but PCR-positive samples from Nchelenge District had evidence of pfhrp2 or pfhrp3 deletions.

To our knowledge, this is the first report suggesting the possibility of pfhrp2 deletions in Zambia, although the level of parasite DNA was lower than those recommended by Parr et al. and limited our ability to make deletion calls. In our study, the range of DNA concentrations of pfhrp2-negative samples was 0.0002–0.0006 ng/μL (approximately 8–24 genomes/μL). However, all PCR protocols used to confirm the DNA concentration and subsequently amplify pfhrp2/3 had a detection limit of 0.0001 ng/μL (~4 genomes/μL), and, therefore, the failure to amplify pfhrp2 in three samples was not likely because of insufficient DNA alone. It seems plausible that pfhrp2-deleted parasites are present in Choma District, Zambia, but further investigation is needed to confirm this observation. Genes encoding PFHRP2 and PFHRP3 share high homology, and detection of the D10 strain of *P. falciparum*

![Diagram](image-url)

**Figure 1.** Workflow for detecting *Plasmodium falciparum* histidine–rich protein 2/3 (pfhrp2/3)-negative parasites.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Year</th>
<th>RDT positivity in the community, % (95% CI)</th>
<th>Prevalence of fever in the community, % (95% CI)</th>
<th>Samples considered for screening</th>
<th>Prevalence of fever among sample, % (95% CI)</th>
<th>Samples with <em>P. falciparum</em> DNA concentration &gt; 0.0001 ng/μL</th>
<th>pfhrp2-negative, number (%; 95% CI)</th>
<th>pfhrp2/3 double negative, number (%; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choma</td>
<td>2009–2011</td>
<td>0.7 (0.4, 1.1)</td>
<td>1.3 (0.8, 1.8)</td>
<td>29</td>
<td>3.4 (0.1, 18)</td>
<td>8</td>
<td>3 (38; 8.5, 76)</td>
<td>0 (0; 0, 37)*</td>
</tr>
<tr>
<td>Nchelenge</td>
<td>2015–2017</td>
<td>45 (43, 47)</td>
<td>3.6 (2.9, 4.3)</td>
<td>40</td>
<td>0 (0, 8.9)*</td>
<td>28</td>
<td>0 (0; 0, 14)*</td>
<td>0 (0; 0, 14)*</td>
</tr>
</tbody>
</table>

* RDT = rapid diagnostic test.
* One-sided, 97.5% CI.

**Table 1** Summary of screening results to detect *Plasmodium falciparum* histidine–rich protein 2/3 (pfhrp2/3)-negative parasites in northern and southern Zambia
**REFERENCES**


