Prevalence of *Plasmodium falciparum* lacking histidine-rich proteins 2 and 3: a systematic review

Rebecca Thomson, Jonathan B Parr, Qin Cheng, Stella Chenet, Mark Perkins & Jane Cunningham

**Objective** To calculate prevalence estimates and evaluate the quality of studies reporting *Plasmodium falciparum* lacking histidine-rich proteins 2 and 3, to inform an international response plan.

**Methods** We searched five online databases, without language restriction, for articles reporting original data on *Plasmodium falciparum*-infected patients with deletions of the *pfhrp2* and/or *pfhrp3* genes (*pfhrp2/3*). We calculated prevalence estimates of *pfhrp2/3* deletions and mapped the data by country. The denominator was all *P. falciparum*-positive samples testing positive by microscopy and confirmed positive by species-specific polymerase chain reaction testing (PCR). If microscopy was not performed, we used the number of samples based on a different diagnostic method or PCR alone. We scored studies for risk of bias and the quality of laboratory methods using a standardized scoring system.

**Findings** A total of 38 articles reporting 55 studies from 32 countries and one territory worldwide were included in the review. We found considerable heterogeneity in the populations studied, methods used and estimated prevalence of *P. falciparum* parasites with *pfhrp2/3* deletions. The derived prevalence of *pfhrp2* deletions ranged from 0% to 100%, including focal areas in South America and Africa. Only three studies (5%) fulfilled all seven criteria for study quality.

**Conclusion** The lack of representative surveys or consistency in study design impairs evaluations of the risk of false-negative results in malaria diagnosis due to *pfhrp2/3* deletions. Accurate mapping and strengthened monitoring of the prevalence of *pfhrp2/3* deletions is needed, along with harmonized methods that facilitate comparisons across studies.

---

### Prevalence of *Plasmodium falciparum* lacking histidine-rich proteins 2 and 3: a systematic review

**Objective** To calculate prevalence estimates and evaluate the quality of studies reporting *Plasmodium falciparum* lacking histidine-rich proteins 2 and 3, to inform an international response plan.

**Methods** We searched five online databases, without language restriction, for articles reporting original data on *Plasmodium falciparum*-infected patients with deletions of the *pfhrp2* and/or *pfhrp3* genes (*pfhrp2/3*). We calculated prevalence estimates of *pfhrp2/3* deletions and mapped the data by country. The denominator was all *P. falciparum*-positive samples testing positive by microscopy and confirmed positive by species-specific polymerase chain reaction testing (PCR). If microscopy was not performed, we used the number of samples based on a different diagnostic method or PCR alone. We scored studies for risk of bias and the quality of laboratory methods using a standardized scoring system.

**Findings** A total of 38 articles reporting 55 studies from 32 countries and one territory worldwide were included in the review. We found considerable heterogeneity in the populations studied, methods used and estimated prevalence of *P. falciparum* parasites with *pfhrp2/3* deletions. The derived prevalence of *pfhrp2* deletions ranged from 0% to 100%, including focal areas in South America and Africa. Only three studies (5%) fulfilled all seven criteria for study quality.

**Conclusion** The lack of representative surveys or consistency in study design impairs evaluations of the risk of false-negative results in malaria diagnosis due to *pfhrp2/3* deletions. Accurate mapping and strengthened monitoring of the prevalence of *pfhrp2/3* deletions is needed, along with harmonized methods that facilitate comparisons across studies.

---

**Introduction**

Despite improvements in malaria control over the past decade, malaria caused an estimated 405,000 deaths worldwide in 2018.1 In 2010, World Health Organization (WHO) treatment guidelines established that all cases of suspected malaria should be confirmed by microscopy or an antigen-detecting rapid diagnostic test before treatment.2 Malaria rapid diagnostic tests contain one or a combination of antibodies that recognize specific plasmodial antigens. These antigens include histidine-rich protein 2 (HRP2) which is specific to *P. falciparum*, and genus- and species-specific lactate dehydrogenase or aldolase, which are produced by all four major human-infecting *Plasmodium* species.3 The number of rapid diagnostic tests procured has increased significantly, from 10 million in 2002 to 412 million in 2018.4 The great majority of these tests detect an HRP2 target, alone or with another antigen, with 15 of 16 (94%) WHO-prequalified malaria tests targeting HRP2 for *P. falciparum* detection.4

Rapid diagnostic tests targeting HRP2 came to dominate the market because they are generally more sensitive than other assays and tend to be more heat stable.5,6 The presence of repetitive epitopes in HRP2 provides numerous antibody binding sites and enables the detection of low levels of protein. The monoclonal antibodies used in HRP2-detecting tests often cross-react with HRP3, encoded by the *pfhrp3* gene,7,8 particularly at parasite counts above 1000 per µL of blood.9 HRP3 is a structural homologue of HRP2 that shares similar amino-acid repeats.10,11 Deletions in the *pfhrp2* and/or *pfhrp3* (*pfhrp2/3*) genes as a cause of false-negative rapid diagnostic tests was first recognized in 2010 in the Peruvian Amazon basin.12 Molecular testing by polymerase chain reaction (PCR) confirmed *P. falciparum* infection, but also that *pfhrp2* and *pfhrp3* genes were deleted in 41% (61 samples) and 70% (103 samples) of these 148 samples, respectively.13 Additional analyses have confirmed a significant increase in the frequency of samples showing *pfhrp2/3* deletions in the same area.12,13 More recently, malaria parasites with *pfhrp2/3* gene deletions have been documented in other parts of the world including East,14 Central,15 West16 and Southern Africa,17 Asia18 and the Middle East.19 Most concerning was a study in Eritrea that reported samples from 62% (31/50) of microscopy-confirmed *P. falciparum* patients testing negative for *pfhrp2*.20 Collectively, these reports suggest a global threat to the continued use of HRP2-based rapid diagnostic tests.

In 2014, recommendations on investigating and accurate reporting of *pfhrp2/3* gene deletions were published.21 Additional criteria have been proposed in more recent studies, including parasite quantification by microscopy or quantitative PCR to rule out false-negative *pfhrp2* detection in samples below the limit of detection of the *pfhrp2* assay,9 and analysis of *pfhrp3*.22 However, we have found no assessments of the uptake of these recommendations.

---

**Abstracts in Arabic, Chinese, English, French, Russian and Spanish at the end of each article.**
There are increasing numbers of reports documenting the threat of mutant parasite genes for malaria case management. However, due to different study designs and laboratory methods it is difficult to compare findings across studies and accurately understand this threat. We aimed to compile all published studies on the prevalence of pfhrp2/3 gene deletions and assess the quality of methods and reporting. We used our findings to paint a global picture of the current status of pfhrp2/3 deletions to guide decisions on the locations and methods of future surveys.

Methods

Search strategy and data extraction

We carried out a systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement.23 We made a search of the online databases of PubMed®, Scopus, LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde), WHO Global Index Medicus and the Web of Science for articles published in any language between 1 January 2010 and 20 August 2019. We used the search terms “[histidine* OR hrp* OR pfhrp*] AND [deletion* OR variation OR diversity OR lack] AND [malaria OR falciparum]” to identify articles reporting molecular analysis of Plasmodium falciparum parasite samples for pfhrp2/3 deletions. Additional articles were identified through manual searches. Further information about the search criteria are provided in Table 1. Two investigators screened the titles and abstracts of all eligible articles and extracted the following information from the full text: country, study sites, study design, year(s) of data collection, patient symptom status, age range, number of Plasmodium falciparum-positive patients, type of blood sample, which samples underwent molecular analysis, number of samples with pfhrp2/3 deletions, laboratory methods (seven items; Box 1) and analysis of flanking genes. Discrepancies in the data were double-checked.

Prevalence estimates

To maximize consistency in calculating prevalence across studies, we used the total number of Plasmodium falciparum samples testing positive by microscopy and confirmed Plasmodium falciparum-positive by species-specific PCR as the denominator. We did this regardless of whether all or only a subset of patient samples were tested for pfhrp2/3 deletion by molecular analysis or whether it was the denominator reported in the original publication. If microscopy was not performed, we used the number of samples based on a different diagnostic method or PCR alone. We did not make prevalence estimates from case reports. All prevalence estimates in this review were therefore derived using a standardized denominator and not necessarily the same prevalence as reported in the original article.

Where researchers collected samples from multiple countries, or used different sampling methods or time frames, we separated the results by country or sample collection group to present prevalence data as separate studies. We presented compiled results for studies which collected samples at one point in time from multiple sites with the same sampling design. When we combined data from different studies by country,

| Table 1. Inclusion and exclusion criteria for selection of studies in the systematic review of Plasmodium falciparum pfhrp2/3 gene deletions |
|---------------------------------|-----------------|-----------------|
| Characteristic                  | Included        | Excluded        |
| Study population                | All ages and populations | None |
| Study outcome                   | Percentage of samples testing negative for pfhrp2 gene, with or without analysis of pfhrp3 gene | Studies which analysed variation in pfhrp2 genetic sequence only |
| Method of confirmation of pfhrp2 and/or pfhrp3 gene deletion | Molecular analysis of pfhrp2/3 gene deletions | Suspected deletions based on rapid diagnostic testing, microscopy or serological testing only |
| Study design                    | All, including case studies, cross-sectional or convenience studies | None |
| Type of paper                   | Published articles of an original study | Review articles, doctoral theses, abstracts with no corresponding published article |
| Patient status                  | Symptomatic suspected malaria patients or asymptomatic people | None |
| Area of data collection         | All countries and regions | None |
| Date of study publication       | 1 January 2010 to 20 August 2019 | Prior to 1 January 2010 |

Box 1. Assessment of study quality in the systematic review of Plasmodium falciparum pfhrp2/3 gene deletions

We assessed a total of seven criteria for quality of laboratory methods, five based on recommendations from previous research22 and two additional criteria. The number and percentage of studies complying with each quality criterion were as follows (n = 55 studies):

- Quality-assured microscopy: 45 studies (82%).
- Plasmodium falciparum species confirmation by PCR test: 55 studies (100%).
- Detection of two other single-copy genes: 21 studies (38%).
- Detection of pfhrp3 gene by PCR test: 46 studies (84%).
- Parasite density quantification: 36 studies (66%).

We awarded one point per criterion satisfied and assigned a total quality score for each study (from 1 to 7), as follows:

Score 1: 0 studies (0%); Score 2: 6 studies (11%); Score 3: 5 studies (9%); Score 4: 16 studies (30%); Score 5: 15 studies (27%); Score 6: 10 studies (18%); Score 7: 3 studies (6%).

HRP2: histidine-rich protein 2; PCR: polymerase chain reaction; WHO: World Health Organization.
we weighted the percentage of samples with pfhrp2 gene deletions to account for differing sample sizes. We used the middle year of the data collection period for studies spanning multiple years.

Assessment of study quality and bias

We assigned a total quality score from 1 to 7 to each study, based on fulfilment of seven criteria for quality of laboratory methods (Box 1).

We assessed study bias as a score from 1 (lowest bias) to 4 (highest bias). The values show the potential bias of the derived prevalence estimate from the true prevalence in the population, depending on the sample population (symptomatic, asymptomatic, mixed or unrepresentative) and samples tested for pfhrp2/3 genes (all, discordant only or another subset). Studies which analysed all samples have a lower bias score than those which only analysed discordant or a subset of samples, while studies which included both symptomatic and asymptomatic samples have a lower study bias than those which only analysed samples from symptomatic people or an unrepresentative sample.

Results

After screening 115 articles, we included 38 articles in the review (Fig. 1).11–20,24–50 Within the articles we identified 55 distinct studies conducted in 32 countries and one territory in the regions of Africa, Americas, South-East Asia and Eastern Mediterranean (Table 2; available at: http://www.who.int/bulletin/volumes/98/8/20-250621).

Study characteristics

The included studies showed substantial differences in study design, laboratory methods and data reporting.

Sample populations

The number of samples tested for pfhrp2 ranged from 1 to 783, while the denominator of P. falciparum-positive samples ranged from 1 to 3291 (Table 2). Out of the 55 studies, 36 (65%) analysed blood samples only from people with symptoms of malaria, as part of a prospective or retrospective survey including unbiased cohorts. Samples in these studies were collected from suspected malaria patients presenting to health facilities or through active case detection. Eight studies (15%) included samples from asymptomatic and symptomatic people as part of cross-sectional surveys or malaria screening programmes, while eight other studies (15%) used samples from an unrepresentative sample of participants and three studies (5%) did not specify the symptom status of the participants. One study collected samples from patients with severe malaria only, while one study collected equal numbers of samples from human immunodeficiency virus-positive and -negative children.

In 35 studies (64%) all samples underwent pfhrp2/3 genotyping. Thirteen studies (24%) genotyped discordant samples only. Of these, nine studies analysed only microscopy-positive and HRP2-rapid diagnostic test-negative samples (of which two were case studies including only one sample), while four studies genotyped only samples which were negative by HRP2-rapid diagnostic test and positive by PCR. One article reporting seven studies only genotyped samples showing the lowest HRP2 concentrations by enzyme-linked immuno sorbent assay.

Study procedures

Only three studies (5%) fulfilled all seven criteria for quality of procedures (Box 1). While the number of P. falciparum-positive samples was based on microscopy- and PCR-positive results in 45 studies (82%), in nine studies (16%) the denominator was based on PCR results alone, and in one study (2%) it was based on P. falciparum-specific lactate dehydrogenase-based rapid diagnostic tests and confirmed by PCR. The presence of P. falciparum was confirmed most commonly by amplification of the multi-copy 18SrRNA gene. Thirty-four studies (62%) analysed samples from dried blood spots, 13 (24%) used venous blood, seven (13%) used a combination of both and one study (2%) did not provide information on sample type. Forty-six studies (84%) conducted molecular analysis to determine pfhrp3 deletion. One of these studies only genotyped pfhrp3 deletions among samples found to be pfhrp2-negative.

Twenty-one studies (38%) did not amplify any other single-copy genes while 13 studies (24%) amplified one other and 21 studies (38%) amplified at least two other single-copy genes. To rule out negative pfhrp2/3 PCR results being due to parasite density below the limit of detection of the assay, only samples which were positive by other single-copy genes and failed to amplify the pfhrp2/3 gene were considered to be pfhrp2/3-deleted in the 21 studies which conducted this analysis. The most commonly selected genes for confirmation were P. falciparum merozoite surface proteins 1 and 2, and glutamate-rich protein. One study confirmed pfhrp2
deletion by testing for pfhrp3. However, while parasite density was measured in 36 studies, only five studies used these results when determining if a sample was pfhrp2/3-negative. In three studies only samples above a chosen parasite density or deoxyribonucleic acid (DNA) concentration were tested for pfhrp2, while in one study all samples below 5 parasites per µL of blood were discounted and in one study samples were only included in the original study if they were above 2000 parasites per µL of blood.

Twenty-nine studies (53%) amplified both exons 1 and 2 of the pfhrp2 gene, while 26 studies (47%) amplified only exon 2 and 28 studies (51%) amplified the flanking genes of pfhrp2. The studies that amplified exon 1 were not necessarily those that amplified flanking genes, with 19 studies (35%) amplifying both exon1 and flanking genes.

**Prevalence estimates**

The derived prevalence of pfhrp2 gene deletions in the 55 studies ranged from 0% to 100% (Table 2). Although we present the overall results by study, 14 studies were conducted over many sites and showed geographically heterogenous results. Further details about the results presented by region are provided in the data repository.31

In Fig. 2 we mapped the geographical distribution of the highest derived prevalence estimate of pfhrp2 gene deletions by study for each country. The highest derived prevalence was above 50% in Colombia, Eritrea and Peru. Fig. 3 plots the weighted average prevalence of pfhrp2 gene deletions for each country and the range by study sites. The weighted average prevalence ranged from 0% to 43%. Average prevalence above 20% was found in Eritrea, Ghana, Nicaragua, Peru and Sudan.

We plotted the prevalence of pfhrp2/3 gene deletions by sample size (available in the data repository).32 Five studies had a sample size over 1000, while 36 had sample sizes smaller than 100. All seven studies reporting greater than 50% prevalence of pfhrp2 deletions had a sample size of fewer than 55. Scatter plots of prevalence against time are available in the data repository.33

**Risk of bias**

Table 2 shows the bias scores of the prevalence estimates from the true prevalence of pfhrp2/3 gene deletions in the population. Six studies (11%) had a bias score of four while two (4%) had a bias score of one.

**Discussion**

We found that mutant parasites have been reported from all major malaria-endemic areas, in asymptomatic and symptomatic *P. falciparum*-positive patients. However, our results also confirm that the full extent of the threat has not yet been characterized. The limited number of well conducted prevalence surveys in malaria-endemic countries indicate geographical variability in the prevalence of mutations in the pfhrp2 and pfhrp3 genes and do not completely illuminate the factors driving these differences.

The study has limitations. Although we included only published articles, we were aware of other abstracts and doctoral theses for which relevant data on methods were not available. For manuscripts included in the review, we contacted authors to obtain information not included in the manuscripts; this was not always possible, however, and we therefore occasionally made assumptions about the methods. Survey design and sample populations varied greatly across the included studies. Most studies were not purposely designed for investigating the prevalence of gene deletions and relied on convenience sampling or on secondary analyses of existing specimens. These shortcomings limit our ability to draw conclusions that can inform the use of rapid diagnostic testing, but rather provides guidance for future surveys.

Reconciling the different populations and sample sizes across studies is challenging. First, studies of asymptomatic and symptomatic patients require different interpretations and are difficult to integrate. Samples from asymptomatic patients may have lower parasite densities, resulting in less DNA target for amplification and potentially greater risk of falsely reporting pfhrp2 deletions. This risk is especially high when the investigation does not include amplification of other single-copy genes or does not quantify parasite DNA. Furthermore, little is known about the effect of pfhrp2/3 gene deletions on the virulence of malaria infection. If pfhrp2/3 deletions are associated with less virulent infections, there could be a difference in prevalence between symptomatic and asymptomatic infections. We found numerous studies with low sample sizes which may not be representative of the true prevalence of deletions in a population or country.

Second, different selection criteria for pfhrp2/3 genotyping (all malaria suspects or only those with discordant HRP2-based rapid diagnostic test and microscopy and/or PCR results) result in the use of different numerators and denominators for prevalence estimation across studies. Analysis limited to deletions found among discordant samples leads to a higher prevalence of gene deletions being reported. To improve consistency in calculating the prevalence of pfhrp2-negative mutants, we used the total number of samples with confirmed pfhrp2 gene deletions by species-specific PCR as the numerator and the total cohort number of *P. falciparum*-positive samples by microscopy and/or PCR as the denominator. The WHO-recommended approach of testing only a subset of high-risk (discordant) samples is a more economical way of monitoring the prevalence of gene deletion and targets clinically significant deletions that cause negative test results. WHO recommends using non-exclusive HRP2-based rapid diagnostic tests when the prevalence of pfhrp2/3 gene deletions causing false-negative test results is greater than 5%.

Most studies included in this review did not allow us to determine if the WHO threshold had been exceeded. It is well acknowledged that the WHO approach may underestimate the prevalence of pfhrp2 deletions in the parasite population. Samples that are pfhrp2-negative and pfhrp3-positive are not likely to be flagged as high risk or discordant due to the cross-reactivity between HRP2 and HRP3 proteins on many rapid diagnostic test brands. However, this concern does not pose an immediate threat to patients.

Ideally, all *P. falciparum*-positive samples should be used as the denominator, determined either by microscopy or a good quality rapid diagnostic test for detecting *P. falciparum*-specific lactate dehydrogenase.

The study bias scores show the potential bias of the prevalence estimates from the true prevalence of pfhrp2/3 gene deletions in the population, but not necessarily the bias of deletions causing false-negative results (which is more important for determining the effect on malaria case management). Ultimately,
Fig. 2. Geographical distribution of highest prevalence estimates for *Plasmodium falciparum* *pfhrp2* gene deletions by study among patients tested at the country level.

Notes: We calculated the prevalence of *pfhrp2* gene deletions using all *Plasmodium falciparum* positive samples as the denominators by country. We used the highest prevalence estimates for each study.
high-throughput screening options could become more readily available and more commonly used. If so, the true prevalence of \( pfhrp2 \) gene deletions could be determined by molecular testing of all people with suspected malaria regardless of rapid diagnostic test or microscopy results, and those samples confirmed to have \( pfhrp2 \) deletions used as the numerator.

Third, recent modelling suggests that the likelihood of finding \( pfhrp2/3 \) deletions can vary during the malaria transmission season due to changes in the transmission intensity and multiplicity of infection, whereby a person can be infected with multiple \( P. falciparum \) strains.\(^5\) Co-infection with \( pfhrp2/3 \)-negative- and wild-type parasites can prevent detection of gene deletions using current laboratory techniques, leading to an underestimation of the prevalence of \( pfhrp2/3 \)-negative mutants. Time of year and relation to the transmission season is rarely described in published reports. A publicly available database using prediction models could be useful to help determine the optimal time in the transmission season to conduct a gene deletion survey.\(^5\)

Due to the small number of studies, differing populations and often small samples sizes of the reviewed studies, it is difficult to draw robust conclusions on the prevalence of \( pfhrp2/3 \) gene deletions in specific areas or to perform meta-analysis from these data. The implementation of more large-scale, robust surveys would enable a better understanding of if, and at what rate, these mutations are increasing in a given area, and would allow for meta-analysis.

Identifying the prevalence of \( pfhrp2/3 \) deletion mutations requires synthesis of several lines of evidence and study procedures that include proper performance of rapid diagnostic tests and careful genotyping methods. While most studies in this review followed some components of published criteria to classify a sample as \( pfhrp2 \)-deleted,\(^2\) only 3 (5\%) of the studies followed the seven recommended criteria proposed in this review. One specific challenge for molecular analyses of \( pfhrp2/3 \) is using the absence of amplified products as the indicator of interest, rather than the presence of amplified products. Rigorous methods and appropriate controls must be used to ensure the presence of
non-degraded, amplifiable parasite DNA and the lack of amplicon contamination. Improving the accuracy of survey outcomes requires novel molecular-based technology and methods that could detect pfhrp2/3 gene deletions more reliably and efficiently and detect pfhrp2/3 deletions in samples with mixed infections (such as quantitative-PCR and whole genome sequencing). Not all malaria-endemic countries have the capacity to conduct molecular analysis to a high standard, and establishing such capacity is challenging and costly. In addition, the sensitivity and specificity of PCR assays can be affected by the protocol used, potentially resulting in variations in the results across laboratories following different procedures. For example, lowering the elongation temperature on pfhrp2 assays improved the limit of detection of many previously published assays.\(^2\) WHO has established a network of laboratories capable of conducting this analysis to ensure that samples from prevalence surveys can be performed quickly and procedures harmonized across laboratories.\(^2\)

Just over half of the studies amplified both exon 1 and 2 of the pfhrp2 gene, while the rest amplified only exon 2. While the chromosomal break points could theoretically be anywhere within the pfhrp2 gene, it appeared that most samples from Eritrea and Peru have observed deletions in both exon 1 and 2 (Qin Cheng, Australian Defence Force Malaria and Infectious Disease Institute, personal communication, 2019). Therefore, whether analysis of exon 2 alone is sufficient to identify most parasites with pfhrp2 gene deletions requires further analysis of gene deletions from other parasite populations. While not included in the recommendations for pfhrp2/3 molecular analysis,\(^2\) analysis of flanking genes can provide additional information on genetic mutations.

Despite the diversity of study approaches, there appear to be areas of high prevalence of pfhrp2/3 mutant parasites where diagnostic testing based on HRP2 alone would be inadequate. Thus, the need for alternative rapid diagnostic tests is of urgent concern in the Amazon basin and Eritrea, where the prevalence of tests producing false-negative results among symptomatic patients has forced changes in the diagnostic strategy.\(^3\) Malaria control programmes should remain vigilant for evidence suggesting the presence of pfhrp2/3 gene deletions. Evidence of false-negative rapid diagnostic tests or confirmed pfhrp2/3-negative mutants in neighbouring countries should trigger careful investigation and surveillance. To improve the quality and relevance of surveys for clinical case management, WHO now provides general guidance on when to prioritize surveys for pfhrp2/3 deletions.\(^2\) WHO has also developed protocols for guiding survey design, data collection and laboratory methods to determine the prevalence of clinically-relevant pfhrp2/3 deletions causing false-negative rapid diagnostic tests.\(^9\) The guidelines aim to ensure that future investigations are implemented to high and comparable standards. Additionally, an up-to-date repository of pfhrp2/3 deletion studies is maintained on the WHO malaria threat map.\(^4\)

The specific factors that drive the evolution and spread of pfhrp2/3 mutations are not clear, although mathematical models suggest that selective pressure by HRP2-detecting rapid diagnostic tests over the past decade is likely to have played an important role.\(^2\) Low malaria transmission and high frequency of people correctly treated on the basis of diagnosis with HRP2-detecting tests have also been identified as key drivers of the selection of pfhrp2/3-negative mutants.\(^9\) Nevertheless, the existence and rising prevalence of pfhrp2 deletions in Peru,\(^11,12,33\) where HRP2-only methods have never been widely used, along with the local prevalence of pfhrp3 mutations, confirms that selective treatment based on test results is not the only factor driving the evolution of these parasites.

Due to the global reliance on rapid diagnostic tests for malaria diagnosis, a coordinated, multifaceted response to P. falciparum with pfhrp2/3 gene deletions is required. This response should include representative studies of the prevalence and distribution of pfhrp2/3 deletions, more efficient and affordable methods for screening and confirming these deletions, and efforts to standardize and ensure high-quality reporting. Follow-up surveys in areas with documented pfhrp2/3 deletions will provide insight into the speed at which the mutant parasites are evolving in response to diagnostic pressure and other drivers. Research for the development and commercialization of rapid diagnostic tests based on new or improved non-HRP2 targets is an essential parallel area of work.

Acknowledgements
We thank Ryan O’Neil Williams and Andrea Bosman.

Funding: This review was funded by The Bill & Melinda Gates Foundation.

Competing interests: None declared.
目的
旨在计算感染率估值并评估报告缺失富组氨酸蛋白 2 和 3 的恶性疟原虫感染率：系统评审

方法
我们在没有语言限制的情况下搜索了五个在线数据库，以获取报告缺失 pfhrp2 和 / 或 pfhrp3 基因（pfhrp2/3）的恶性疟原虫感染患者原始数据的文章。我们计算了缺失 pfhrp2/3 的感染率估值并按国家映射数据。分母是所有经显微镜检测均为阳性并通过种特异性聚合酶链反应试验（PCR）确认为阳性的恶性疟原虫阳性样本。如果进行显微镜检测，我们采用基于不同诊断方法或单独 PCR 的方法。我们使用标准化评分系统对偏倚风险以及实验室方法的质量研究进行了评分。

结果
评审中共纳入 38 篇文章，这些文章报告了全球 32 个国家和 1 个地区中的 55 项研究。在研究的人群、使用的方法以及估计的缺失 pfhrp2/3 的恶性疟原虫感染率中，我们发现相当大的异质性。推导的 pfhrp2 缺失感染率介于 0% 至 100% 之间，包括南美洲和非洲的重点地区。就研究质量而言，只有三项研究 (5%) 符合所有七个标准。

结论
缺乏代表性调查或研究设计缺乏一致性妨碍了由于 pfhrp2/3 缺失导致疟疾诊断中假阴性结果的风险的评估。需要准确映射和加强对 pfhrp2/3 缺失感染率的监测，以及促进各项研究比较的统一方法。

Résumé
Prévalence de Plasmodium falciparum sans protéines 2 et 3 riches en histidine: revue systématique

Objectif
Estimer la prévalence et évaluer la qualité des études consacrées à Plasmodium falciparum sans protéines 2 et 3 riches en histidine afin d'établir un plan d'intervention international.

Méthodes
Nous avons parcouru cinq bases de données en ligne sans restriction de langue pour trouver des articles contenant des informations d’origine relatives à des patients atteints de Plasmodium falciparum dépourvu des gènes pfhrp2 et/ou pfhrp3 (pfhrp2/3). Nous avons calculé la prévalence des délétions des gènes pfhrp2/3 et cartographié les données par pays. Le dénominateur était représenté par les échantillons positifs à P. falciparum, testés positifs au microscope et confirmés par un test de réaction en chaîne par polymérase (PCR) propre à l’espèce. Si aucun examen n’avait été effectué au microscope, nous avons utilisé le nombre d’échantillons recourant à une méthode de diagnostic différente, ou uniquement à la PCR. Nous avons noté les études selon le risque de biais et la qualité des techniques d’analyse en laboratoire, à l’aide d’un système de notation standardisé.

Résultats
Au total, 38 articles mentionnant 55 études réalisées dans 32 pays et un territoire dans le monde ont été pris en compte dans cette revue. Nous avons observé une grande hétérogénéité dans les populations étudiées, les méthodes employées et la prévalence estimée des parasites P. falciparum assortis d’une délétion des gènes pfhrp2/3. La prévalence dérivée des délétions de pfhrp2 est comprise entre 0% et 100%, avec des zones de convergence en Amérique du Sud et en Afrique. Seules trois études (5%) remplissaient l’ensemble des sept critères de qualité.

Conclusion
L'absence d'enquêtes représentatives ou d'uniformité dans la conception des études empêche d'évaluer correctement le risque de faux négatifs dans le diagnostic de la malaria en raison des délétions de pfhrp2/3. Une cartographie détaillée ainsi qu’une surveillance renforcée de la prévalence des délétions de pfhrp2/3 est nécessaire, tout comme une harmonisation des méthodes afin de faciliter la comparaison entre les différentes études.

Резюме
Распространенность Plasmodium falciparum с отсутствием богатых гистидин белков 2 и 3: систематический обзор

Цель
Вычислить распространенность и оценить качество исследований, посвященных распространенности Plasmodium falciparum с отсутствием богатых гистидин белков 2 и 3, с целью получения информации для разработки международного плана реагирования.

Методы
Авторы выполнили поиск статей, содержащих исходные данные по пациентам, зараженным Plasmodium falciparum с дефектами генов pfhrp2 и/или pfhrp3 (pfhrp2/3), в пяти базах данных в Интернете на разных языках. Авторы выполнили оценку распространенности дефектов pfhrp2/3 и составили карту данных для разных стран. В качестве знаменателя использовалось общее количество проб на Plasmodium falciparum с положительным результатом, полученным при микроскопическом исследовании и подтвержденным в ходе видоспецифического тестирования методом полимеразной цепной реакции (ПЦР). Если микроскопическое исследование не проводилось, авторы использовали данные о количестве проб, полученные на основании другого диагностического метода или только на основании ПЦР. Исследования оценивались по уровню риска необъективности и качеству лабораторных методов с применением стандартной системы оценок.
Resumen

Prevalencia del *Plasmodium falciparum* que carece de las proteínas 2 y 3 en histidina: un análisis sistemático

Objetivo: Calcular las estimaciones de la prevalencia y evaluar la calidad de los estudios que informan de la existencia del *Plasmodium falciparum* que carece de las proteínas 2 y 3 en histidina, para elaborar un plan de respuesta internacional.

Métodos: Se revisaron cinco bases de datos en línea, sin restricción de idioma, para encontrar artículos que informaran sobre los datos originales de los pacientes infectados con *Plasmodium falciparum* con delecciones de los genes pfhrp2 y pfhrp3 (pfhrp2/3). Se calcularon las estimaciones de prevalencia de las delecciones de pfhrp2/3 y se clasificaron los datos por país. El denominador eran todas las muestras positivas por *P. falciparum* que daban positivo en las pruebas de microbiología y confirmadas como positivas en las pruebas de reacción en cadena de la polimerasa (PCR, por sus siglas en inglés) específicas de la especie. Si no se realizaba la microbiología, se empleaba el número de muestras en base a un método de diagnóstico diferente o a la PCR únicamente. Los estudios se calificaron en función del riesgo de sesgo y de la calidad de los métodos de laboratorio por medio de un sistema de puntuación estandarizado.

Resultados: El análisis incluyó un total de 38 artículos en los que se informaba de 55 estudios de 32 países y un territorio a nivel mundial. Se observó una heterogeneidad considerable en las poblaciones estudiadas, los métodos aplicados y la prevalencia estimada de los parásitos *P. falciparum* con delecciones de los genes pfhrp2/3. La prevalencia que se estimó de las delecciones del gen pfhrp2 osciló entre el 0 % y el 100 %, incluidas las áreas focales de América del Sur y África. Tan solo tres estudios (5 %) cumplieron los siete criterios de calidad del estudio.

Conclusión: La falta de encuestas fiables o de consistencia en el diseño de los estudios dificulta las evaluaciones del riesgo de resultados falsos negativos en el diagnóstico de la malaria debido a las delecciones de los genes pfhrp2/3. Se necesita un mapeo preciso y un seguimiento reforzado de la prevalencia de las delecciones de los genes pfhrp2/3, junto con métodos estandarizados que faciliten las comparaciones entre los estudios.

References

1. World malaria report 2019. Geneva: World Health Organization, 2019. Available from: https://www.who.int/publications-detail/world-malaria-report-2019 [cited 2020 May 10].
2. Guidelines for the treatment of malaria. 3rd ed. Geneva: World Health Organization, 2015. Available from: https://www.who.int/malaria/publications/atoz/9789241549127/en/ [cited 2020 Dec 14].
3. World malaria report 2017. Geneva: World Health Organization, 2017. Available from: https://www.who.int/malaria/publications/world-malaria-report-2017/en/ [cited 2019 Sep 15].
4. Interactive guide for high-quality malaria rapid diagnostic test selection. Geneva: Foundation for Innovative Diagnostics, 2018 Available from: https://www.finddx.org/malaria/interactive-guide/ [cited 2019 Sep 15].
5. Gatton ML, Dunn J, Chaudhry A, Cicetti S, Cunningham J, Cheng Q. Implications of parasites lacking *Plasmodium falciparum* histidine-rich protein 2 on malaria morbidity and control when rapid diagnostic tests are used for diagnosis. J Infect Dis. 2017 04 1;215(7):1156–66. doi: http://dx.doi.org/10.1093/infdis/jix094 PMID: 28320343
6. Chiidiom PI, Bowers K, Jorgensen P, Barnwell JW, Grady KK, Luchavez J, et al. The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. Trans R Soc Trop Med Hyg. 2007 Apr;101(4):351–7. doi: http://dx.doi.org/10.1016/j.trstmh.2006.09.007 PMID: 17212967
7. Baker L, McCarthy J, Gatton M, Kyle DE, Bell D, Luchavez J, et al. Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (pfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis. 2005 Sep 1;192(5):870–7. doi: http://dx.doi.org/10.1086/431910 PMID: 16088837
8. Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, et al. Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. J Clin Microbiol. 2006 Aug;44(8):2773–8. doi: http://dx.doi.org/10.1128/JCM.02537-05 PMID: 16901491
9. Beschir KE, Sepulveda N, Bhamal J, Robinson A, Mwangi J, Busula AO, et al. *Plasmodium falciparum* parasites with histidine-rich protein 2 (pfhrp2) and pfhrp3 gene deletions in two endemic regions of Kenya. Sci Rep. 2017 Nov 17;7(1):14718. doi: http://dx.doi.org/10.1038/s41598-017-13031-2 PMID: 29116127
10. Welmers TE, Howard RJ. Homologous genes encode two distinct histidine-rich proteins in a cloned isolate of *Plasmodium falciparum*. Proc Natl Acad Sci USA. 1986 Aug;83(16):6065–9. doi: http://dx.doi.org/10.1073/pnas.83.16.6065 PMID: 30167411
11. Gamboa D, Ho MF, Bendezu J, Torres K, Chiiodini PL, Barnwell JW, et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS One. 2010 01 25;5(1):e8091. doi: http://dx.doi.org/10.1371/journal.pone.0008091 PMID: 20111602
12. Malha J, Gamboa D, Bendezu J, Sanchez L, Crops L, Gillet P, et al. Rapid diagnostic tests for malaria diagnosis in the Peruvian Amazon: impact of pfhrp2 gene deletions and cross-reactions. PLoS One. 2012;7(8):e43094. doi: http://dx.doi.org/10.1371/journal.pone.0043094 PMID: 2295263
13. Akinyi S, Hayden T, Gamboa D, Torres K, Bendezu J, Abdallah JF, et al. Multiple genetic origins of histidine-rich protein 2 gene deletion in *Plasmodium falciparum* parasites from Peru. Sci Rep. 2013 09 30;3(1):2797. doi: http://dx.doi.org/10.1038/srep02797 PMID: 24077522
14. Kozycki CT, Umulisa N, Rulisa S, Mwikarago EI, Musabyimana JP, et al. False-negative malaria rapid diagnostic tests in Rwanda: impact of *Plasmodium falciparum* isolates lacking hrp2 and declining malaria transmission. Malar J. 2017 03 20;16(1):123. doi: http://dx.doi.org/10.1186/s12936-017-1668-1 PMID: 28320390
15. Parr JB, Verity R, Doctor SM, Janko M, Carey-Ewold K, Turman BJ, et al. Pfhrp2-deleted *Plasmodium falciparum* parasites in the Democratic Republic of the Congo: a national cross-sectional survey. J Infect Dis. 2017 07 12;216(1):36–44. PMID: 28177502
48. Li P, Xing H, Zhao Z, Yang Z, Cao Y, Li W, et al. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 in the China-Myanmar border area. Acta Trop. 2015 Dec;152:26–31. doi: http://dx.doi.org/10.1016/j.actatropica.2015.08.003 PMID: 26297799

49. Kumar N, Panda V, Bhatt RM, Shah NK, Mishra N, Srivastava B, et al. Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. Acta Trop. 2013 Jan;125(1):119–21. doi: http://dx.doi.org/10.1016/j.actatropica.2012.09.015 PMID: 23041541

50. Pati P, Dhangadamajhi G, Bal M, Ranjit M. High proportions of pfhrp2 gene deletion and performance of HRP2-based rapid diagnostic test in Plasmodium falciparum field isolates of Odisha. Malar J. 2018 Oct 29;17(1):394. doi: http://dx.doi.org/10.1186/s12936-018-2502-3 PMID: 3073573

51. Thomson R, Parr J, Cheng Q, Chenet S, Perkins M, Cunningham J. Supplementary webappendix: Annex 1 [data repository]. London: figshare, 2019. doi: http://dx.doi.org/10.6084/m9.figshare.12443663 doi: http://dx.doi.org/10.6084/m9.figshare.12443663

52. Thomson R, Parr J, Cheng Q, Chenet S, Perkins M, Cunningham J. Supplementary webappendix: Supplementary figure 1 [data repository]. London: figshare, 2019. doi: http://dx.doi.org/10.6084/m9.figshare.12443660 doi: http://dx.doi.org/10.6084/m9.figshare.12443660

53. Thomson R, Parr J, Cheng Q, Chenet S, Perkins M, Cunningham J. Supplementary webappendix: Supplementary figure 2 [data repository]. London: figshare, 2019. doi: http://dx.doi.org/10.6084/m9.figshare.12443651 doi: http://dx.doi.org/10.6084/m9.figshare.12443651

54. Malaria rapid diagnostic test performance: results of WHO product testing for pfhrp2- and pfhrp3-negative Plasmodium falciparum. Malar J. 2018 Apr 2;17(1):105. doi: http://dx.doi.org/10.1186/s12936-018-2287-4 PMID: 29609602

55. Watson OJ, Verity R, Ghani AC, Garske T, Cunningham J, Tshefu A, et al. Impact of seasonal variations in Plasmodium falciparum malaria transmission on the surveillance of pfhrp2 gene deletions. eLife. 2019 Oct 5;e40339. doi: http://dx.doi.org/10.7554/eLife.40339 PMID: 31045490

56. Watson OJ. Seasonal impacts on pfhrp2-deletions [internet]. Boston: Rstudio; 2018. Available from: https://ojwatson.shinyapps.io/seasonal_hrp2/ [cited 2018 Oct 21].

57. Parr JB, Anderson O, Juliano JJ, Meshnick SR. Streamlined, PCR-based testing for pfhrp2- and pfhrp3-negative Plasmodium falciparum. Malar J. 2018 Mar 4;17(1):137. doi: http://dx.doi.org/10.1186/s12936-018-2287-4 PMID: 29609602

58. Berhane A, Russom M, Bahta I, Hagos F, Ghirmai M, Uqubay S. Rapid diagnostic tests failing to detect Plasmodium falciparum infections in Eritrea: an investigation of reported false negative rapid diagnostic test results. Malar J. 2017 Oct 3;16(1):105. doi: http://dx.doi.org/10.1186/s12936-017-1752-9 PMID: 28264689

59. Protocol for estimating the prevalence of pfhrp2/pfhrp3 gene deletions among symptomatic falciparum patients with false-negative rapid diagnostic test results. Geneva: World Health Organization; 2018. Available from: http://www.who.int/malaria/publications/atoz/hrp2-deletion-protocol/en/ [cited 2020 May 12].

60. Malaria threat maps [internet]. Geneva: World Health Organization; c2020. Available from: http://apps.who.int/malaria/maps/threats/ [cited 2020 May 12].

61. Watson OJ, Slater HC, Verity R, Parr JB, Mwandagalirwa MK, Tshefu A, et al. Modelling the drivers of the spread of Plasmodium falciparum hrp2 gene deletions in sub-Saharan Africa. eLife. 2017 Oct 24;6:e25008. doi: http://dx.doi.org/10.7554/eLife.25008 PMID: 28837020
### Table 2. Studies reporting pfhrp2 and pfhrp3 gene deletions and derived prevalence estimates by region in the systematic review of *Plasmodium falciparum* pfhrp2/3 gene deletions

| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample population³ | Samples tested⁴ | Total no. of *P. falciparum* positive patients⁵ | No. (%) of samples with gene deletions⁶ | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|------------------|----------------------|-------------------------|-------------|--------------|---------------------|----------------|-----------------------------------------------|----------------------------------------|---------------------------------------------|--------------|-----------------|
| **Africa**       |                      |                         |             |              |                     |                |                                               |                                        |                                             |              |                 |
| Koita et al., 2012³⁵ | Mali               | 1996                    | Sirakoro, Bancoumana, Doneguebougou, Bamako | Cross-sectional | Mixed Discordan t | 480            | 10¹ (2)                                      | NA                                      | NA                                          | NA           | 3               | 3             |
| Ramutton et al., 2012³⁵ | Democratic Republic of the Congo | 2005–2010               | Kinshasa | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 6¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | Gambia            | 2005–2010               | Banjul  | Health facility (Antimalarial drug trial) | Unrepresentative Subsample | 2¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | Kenya             | 2005–2010               | Kilifi  | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 12¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | Mozambique        | 2005–2010               | Beira   | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 19¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | Rwanda            | 2005–2010               | Kigali, Nyanza | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 15¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | United Republic of Tanzania | 2005–2010               | Teule, Korogwe | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 18¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Ramutton et al., 2012³⁵ | Uganda            | 2005–2010               | Mbarare | Health facility (antimalarial drug trial) | Unrepresentative Subsample | 5¹ | 0 (0)                                      | 0 (0)                                    | 0 (0)                                    | NA          | 5               | 2             |
| Wurtz et al., 2013³⁵ | Senegal           | 2009–2012               | Dakar   | Health facility (antimalarial drug trial) | Symptomatic All  | 125 | 3 (2)                                      | 16 (13)                                  | NA                                      | NA          | 5               | 2             |
| Laban et al., 2015³⁵ | Zambia            | 2008–2012               | Choma   | Cross-sectional | Mixed All | 61          | NA                                          | NA                                      | 12¹ (20)                                  | NA          | 4               | 1             |
| Amoah et al., 2016³⁵ | Ghana             | 2015                    | Abura Dunkwa, Obom | Malaria screening programme | Mixed All | 288          | 76 (26)                                     | 85 (30)                                  | 37 (13)                                  | 22–40       | 6               | 1             |

(continues . . )
| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample population<sup>a</sup> | Samples tested<sup>b</sup> | Total no. of P. falciparum positive patients<sup>c</sup> | No. (%) of samples with gene deletions<sup>d</sup> | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|------------------|----------------------|-------------------------|-------------|--------------|-----------------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|----------------|----------------|
| Parr et al., 2017<sup>15</sup> | Democratic Republic of the Congo | 2013–2014 | Kinshasa, Kwango, Kwilu, Mai-Ndombe, Kongo Central, Equateur, Mongala, Nord-Ubangi, Sud-Ubangi, Tshuapa, Kasai, Kasai-Central, Kasai-Oriental, Lomami, Sankuru, Haut-Katanga, Haut-Lomami, Luampa, Tanganyika, Maniema, Nord-Kivu, Bas-Uele, Haut-Uele, Ituri, Tshopo, Sud-Kivu | Cross-sectional | Mixed | Discordant | 2752<sup>i</sup> | 149<sup>i</sup> (5) | NA | 5 (< 1) | 0–22 | 5 | 3 |
| Beshir et al., 2017<sup>9</sup> | Kenya | 2014 | Mbita | Cross-sectional in schools (mosquito behaviour study) | Asymptomatic | All | 131 | 8 (6) | 1.1 | 1 (1) | 0 (0) | NA | 6 | 2 |
| Beshir et al., 2017<sup>9</sup> | Kenya | 2007–2008 | Kilifi | Health facility | Symptomatic | All | 49 | 1 (2) | 1 (2) | 0 (0) | NA | 4 | 2 |
| Gupta et al., 2017<sup>17</sup> | Mozambique | 2010–2015 | Manhiça, Magude | Cross-sectional | Mixed | Discordant | 1162 | 1 (< 1) | NA | 0 (0) | NA | 4 | 3 |
| Kozycki et al., 2017<sup>14</sup> | Rwanda | 2014–2015 | Busogo, Kiribizi, Bukara | Health facility | Symptomatic | Discordant | 3291 | 32<sup>i</sup> (1) | NA | NA | NA | 4 | 4 |
| Menegon et al., 2017<sup>15</sup> | Eritrea | 2013–2014 | Gash Barka, Debub | Unknown | Unknown | All | 144 | 14 (10) | 6 (43) | 13 (9) | 9–22 | 4 | 2 |
| Ranadive et al., 2017<sup>37</sup> | Eswatini | 2012–2014 | Lubombo | Health facility | Symptomatic | Discordant | 162<sup>i</sup> | 0 (0) | 1 (1) | 0 (0) | NA | 4 | 2 |
| Berhane et al., 2018<sup>20</sup> | Eritrea | 2016 | Northern Red Sea, Anseba, Gash Barka, Debub | Health facility | Symptomatic | All | 50 | 31 (62) | 41 (82) | 31 (62) | 42–81 | 7 | 2 |

* (continued...)
| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample population | Samples tested | Total no. of P. falciparum positive patients | No. (%) of samples with gene deletions | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|------------------|---------------------|-------------------------|------------|-------------|-------------------|---------------|------------------------------------------|--------------------------------------|---------------------------------------------|-------------|-----------------|
| Nderu et al., 2018<sup>39</sup> | Kenya | 2007–2016 | Busia, Mbita, Nyando, Tiwi, Msambweni | Drug efficacy trial | Symptomatic | All | 400 | 0 (0) | 0 (0) | NA | 5 | 2 |
| Owusu et al., 2018<sup>38</sup> | Ghana | 2015 | Greater Accra, Eastern region | Cross-sectional study among patients attending antiretroviral therapy clinics | Unrepresentative | Discordant | 62 | 6<sup>n</sup>(10) | 8 (13) | 6 (10) | NA | 4 | 3 |
| Willie et al., 2018<sup>36</sup> | Madagascar | 2014–2015 | Yurimaguas | Health facility | Symptomatic | All | 73 | 0 (0) | NA | NA | NA | 3 | 2 |
| Funwei et al., 2019<sup>41</sup> | Nigeria | 2013–2014 | Elata Ibadan | Health facility | Symptomatic | Discordant | 340 | 11<sup>n</sup>(3) | 4 (1) | 11 (3) | NA | 6 | 4 |
| Kobayashi et al., 2019<sup>42</sup> | Zambia | 2009–2011 | Choma | Cross-sectional | Mixed | Discordant | 45 | 3<sup>n</sup>(7) | NA | 0 (0) | NA | 5 | 3 |
| Kobayashi et al., 2019<sup>42</sup> | Zambia | 2015–2017 | Nchelenge | Cross-sectional | Mixed | Discordant | 1144 | 0<sup>p</sup>(0) | NA | 0 (0) | NA | 6 | 3 |
| Americas | | | | | | | | | | | | |
| Gamboa et al., 2010<sup>13</sup> | Peru | 2003–2007 | Iquitos area, Loreto, Amazonas, Cajamarca | Health facility | Unknown | All | 148 | 61 (41) | 103 (70) | 31 (22) | 36–100 | 4 | 2 |
| Gamboa et al., 2010<sup>13</sup> | Peru | 2007 | Iquitos | Active case detection survey | Symptomatic | All | 9 | 8 (90) | 6 (67) | 4 (44) | NA | 7 | 2 |
| Houzé et al., 2011<sup>25</sup> | Brazil | 2011 | Amazon region | Case study | Symptomatic | Discordant | 1 | 1<sup>n</sup> | 1<sup>n</sup> | 1<sup>n</sup> | NA | 5 | 4 |
| Maltha et al., 2012<sup>12</sup> | Peru | 2010–2011 | Iquitos area | Health facility, active case detection | Symptomatic | All | 74 | 19 (26) | 34 (44) | 19 (26) | NA | 6 | 2 |
| Akinyi et al., 2013<sup>31</sup> | Peru | 1998–2001 | Loreto, Piura | Unknown | Symptomatic | All | 92<sup>0</sup> | 19 (21) | NA | NA | 0–36 | 2 | 2 |
| Akinyi et al., 2013<sup>31</sup> | Peru | 2003–2005 | Iquitos | Unknown | Symptomatic | All | 96<sup>c</sup> | 39 (41) | NA | NA | NA | 2 | 2 |

(continues . . .)
| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample population | Samples tested | Total no. of P. falciparum positive patients | No. (%) of samples with gene deletions | Pfhrp2 | Pfhrp3 | Pfhrp2 and 3 | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|-----------------|----------------------|-------------------------|-------------|--------------|-------------------|---------------|------------------------------------------|----------------------------------------|--------|--------|---------|---------------------------------|--------------|----------------|
| Trouvay et al., 2013 | French Guiana | 2009 | St Luarent du Maroni, Cayenne, St Georges de l'Oyapack, Saul, Antecume Pata | Unknown | Symptomatic | All | 140 | 0 (0) | 4 (3) | 0 (0) | NA | 2 | 2 |
| Trouvay et al., 2013 | French Guiana | 2010-2011 | Cayenne Hospital | Health facility survey | Symptomatic | All | 81 | 0 (0) | 6 (7) | 0 (0) | NA | 5 | 2 |
| Abdallah et al., 2015 | Honduras | 2008-2009 | Puerto Lempira | Health facility survey | Symptomatic | All | 68 | 0 (0) | 30 (44) | 0 (0) | NA | 2 | 2 |
| Akinyi Okoth et al., 2015 | Guyana | 2009-2011 | Georgetown (Cuyuni-Mazaruni, Potaro-Siparuni) | Health facility survey | Symptomatic | All | 97 | 0 (0) | 0 (0) | 0 (0) | NA | 3 | 2 |
| Akinyi Okoth et al., 2015 | Suriname | 2009-2011 | Sipaliwini, Brokopondo | Health facility survey, active case detection | Symptomatic | All | 78 | 11 (14) | 3 (4) | 2 (3) | 0-48 | 3 | 2 |
| Baldeviano et al., 2015 | Peru | 2010-2012 | Tumbes | Health facility during malaria outbreak | Symptomatic | All | 54 | 54 (100) | NA | NA | NA | 2 | 2 |
| Murillo Solano et al., 2015 | Colombia | 2008-2009 | Cordoba, Narino, Valle del Cauca, Amazonas, Guaviare, Meta Esmeeldas | Unknown | Symptomatic | All | 75 | 4 (5) | 40 (53) | 4 (5) | 0-33 | 6 | 2 |
| Murillo Solano et al., 2015 | Colombia | 1999-2007 | Amazonas, Guaviare, Meta | Epidemiological studies | Symptomatic | All | 25 | 14 (56) | 12 (48) | 9 (36) | 0-67 | 4 | 2 |
| Sáenz et al., 2015 | Ecuador | 2012-2013 | Esmeeldas | Malaria outbreak surveillance | Symptomatic | All | 32 | 1 (3) | 1 (3) | 1 (3) | NA | 4 | 2 |
| Dorado et al., 2016 | Colombia | 2003-2010 | Antiqua, Amazonas, Guaviare, Narino, Choco, Cauca, Valle | Unknown | Symptomatic | All | 253 | 15 (6) | 106 (42) | 15 (6) | 0-54 | 4 | 2 |
| Dorado et al., 2016 | Colombia | 2011-2012 | Antiqua, Amazonas, Guaviare, Narino, Choco, Cauca, Valle | Health facility survey | Symptomatic | All | 112 | 0 (0) | 51 (42) | 0 (0) | NA | 6 | 2 |

(continues...)
| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample population | Samples tested | Total no. of P. falciparum positive patients | No. (%) of samples with gene deletions | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|------------------|----------------------|-------------------------|-------------|--------------|------------------|---------------|-----------------------------|-----------------|--------------------------------|-------------|-----------------|
| Okoth et al., 2016 | Peru | 2013 | Cusco | Outbreak surveillance | Symptomatic | All | 4 | 4 (100) | 4 (100) | NA | 4 | 2 |
| Rachid Viana et al., 2017 | Bolivia (Plurinational State of) | 2010–2012 | Beni department | Health facility survey | Symptomatic | All | 25 | 1 (4) | 17 (68) | 0 (0) | NA | 3 | 2 |
| Rachid Viana et al., 2017 | Brazil | 2010–2012 | Acre, Para, Rondonia, Escuintla | Health facility survey | Symptomatic | All | 198 | 27 (14) | 71 (36) | 43 (23) | 0–32 | 4 | 2 |
| Fontecha et al., 2018 | Guatemala | 2015 | Escuintla | Malaria surveillance survey | Symptomatic | All | 21 | 3 (14) | 19 (91) | 3 (14) | NA | 4 | 2 |
| Fontecha et al., 2018 | Honduras | 2011–2017 | Gracias a Dios, Colon, Antántida, Cortes, Islas de la Bahia | Health facility survey for drug resistance | Symptomatic | All | 52 | 13 (25) | 50 (96) | 13 (25) | 0–40 | 4 | 2 |
| Fontecha et al., 2018 | Nicaragua | 2015 | North Atlantic Autonomous Region | Malaria surveillance survey | Symptomatic | All | 55 | 17 (31) | 48 (87) | 11 (20) | NA | 4 | 2 |
| South-East Asia | India | 2010 | Chhattisgarh | Unknown | Symptomatic | All | 48 | 2 (4) | 2 (4) | 2 (4) | NA | 6 | 2 |
| Li et al., 2015 | China–Myanmar border, Thailand | 2011–2012 | China, Myanmar border and Tak province, Thailand | Mass blood survey, unknown | Unknown | All | 97 | 4 (4) | 3 (3) | 3 (3) | NA | 5 | 2 |
| Bharti et al., 2016 | India | 2014 | Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, Maharashtra, Rajasthan, Gujarat, Tripura | Health facility | Symptomatic | Discordant | 152 | 36 (2) | 27 (2) | 25 (2) | 0–25 | 6 | 4 |
| Nima et al., 2017 | Bangladesh | 2013 | Sylhet | Case study | Symptomatic | Discordant | 1 | 1 | 1 | 1 | NA | 5 | 4 |
| Pari et al., 2018 | India | 2013–2016 | Odisha | Cross-sectional | Symptomatic | Discordant | 384 | 38 (10) | 24 (6) | 17 (4) | 8–14 | 7 | 4 |
**Systematic reviews**

Malaria parasites, prevalence and distribution

Rebecca Thomson et al.

### Region and study

| Region and study | Country or territory | Year of data collection | Study sites | Study design | Sample populationa | Samples testedb | Total no. of P. falciparum positive patientsc | No. (%) of samples with gene deletions | Range of pfhrp2 deletion prevalence across study sites | Quality score | Study bias score |
|------------------|----------------------|-------------------------|-------------|--------------|--------------------|----------------|---------------------------------|---------------------------------|---------------------------------|-------------|---------------|
| Atroosh et al., 2015 | Yemen | 2014 | Hodeidah, Al-Mahwit | Active case detection | Symptomatic | All | 189 | 9 (5) | NA | NA | NA | 4 | 2 |
| Mussa et al., 2019 | Sudan | Unrepresentative | Omdurman Health facility | Symptomatic | All | 26 | 9 (39) | NA | NA | NA | 2 | 2 |

DNA: deoxyribonucleic acid; HRP2: histidine-rich protein 2; NA: not applicable; PCR: polymerase chain reaction.

a  Symptomatic: only symptomatic people tested; Mixed: mix of symptomatic and asymptomatic people tested; Asymptomatic: only asymptomatic people tested; Unrepresentative: a subset of people not representative of the population were tested; Unknown: not reported.

b  All: all samples underwent molecular analysis; Discordant: only discordant samples tested; Subsample: another subset of samples tested.

c  As microscopy was not performed, we used the number of *Plasmodium falciparum*-positive cases by PCR as the denominator.

d  Only 22 samples which were rapid diagnostic test-negative and microscopy-positive samples were analysed for *pfhrp2* deletion.

Notes: We calculated the prevalence of gene deletions using all *Plasmodium falciparum*-positive samples as the denominators. All studies used microscopy with PCR confirmation, except where indicated. In some cases, only rapid diagnostic test-negative, microscopy-positive samples or rapid diagnostic test-negative, PCR-positive samples were analysed for *pfhrp2* gene deletion, as indicated in footnotes above. We scored study quality from 1 (lowest) to 7 (highest), as described in Box 1, and study bias from 1 (lowest) to 4 (highest).