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Review Article

Evaluation of PCR-based surveillance as a Prognostic Tool in malaria

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Abstract

Background: Light microscopy and antigen-based rapid diagnostic tests remain the mainstay of malaria diagnosis, but both carry a detection floor of roughly 50-200 parasites/microliter and miss a substantial fraction of infections that polymerase chain reaction (PCR) based methods can resolve [1,2].

Objective: This review synthesizes the published evidence on PCR-based detection and quantification of *Plasmodium* species as a surveillance tool, and on whether PCR-derived parasite load carries prognostic value for disease severity.

Methods: We synthesized assay-development and validation studies, field comparisons of microscopy, rapid diagnostic tests and PCR, systematic reviews of submicroscopic infection, and clinical studies relating parasite DNA load to malaria severity, as a narrative synthesis of the 1993-2018 diagnostics literature.

Findings: PCR consistently recovers infections missed by microscopy and rapid tests, with microscopy-detected *Plasmodium vivax* prevalence averaging 67% lower than PCR-detected prevalence in a 40-study review [8]; plasma parasite DNA concentration was approximately 40-fold and 10-fold higher in severe than uncomplicated falciparum malaria among African children and Asian adults respectively [13]; and submicroscopic carriage becomes proportionally more important as transmission intensity falls [15,16].

Conclusions: PCR-based methods function both as a more sensitive surveillance tool than microscopy or rapid diagnostic testing and, through quantitative parasite load, as a prognostic marker for severe malaria, though infrastructure and cost barriers still limit routine use outside reference and research settings [20,22].

Keywords: malaria; polymerase chain reaction; molecular surveillance; parasitemia; diagnostic sensitivity

1. Introduction

Malaria diagnosis has depended for more than a century on the microscopic examination of a stained blood film, and light microscopy remains the field reference against which every newer method is still measured. Its limitations, however, are well documented: a competent microscopist working a thick film under good conditions rarely resolves parasitemia below roughly 50-100 parasites/microliter, mixed-species infections are frequently misclassified, and inter-reader agreement degrades sharply outside reference laboratories [1,2]. Rapid diagnostic tests built on histidine-rich protein 2 or lactate dehydrogenase antigens addressed the need for a result at the point of care without a trained microscopist, but they inherit a broadly similar detection floor and carry their own failure modes, including antigen persistence after parasite clearance and, in some regions, parasites that have lost the *pfrp2/3* genes the most widely used tests depend on [3,4].

Polymerase chain reaction assays, first adapted to *Plasmodium* species identification in the early 1990s, close much of that gap. Nested PCR targeting the small-subunit ribosomal RNA genes distinguishes the four classically recognized human malaria species from a single field blood spot and resolves mixed infections that microscopy consistently under-calls [5], and a refinement of the same nested strategy pushed analytical sensitivity down to roughly one to five parasites per microliter, an order of magnitude below the microscopic threshold [6]. Three decades on, PCR-based methods have moved from a confirmatory research tool used mainly to adjudicate discordant microscopy results into something closer to a surveillance instrument in its own right, one capable of quantifying parasite burden well below what any antigen- or morphology-based method can see, and of returning that quantity as a number rather than a threshold call.

That shift matters for prognosis as much as for diagnosis. Because circulating parasite DNA, and by extension

quantitative PCR, tracks total parasite burden more closely than peripheral blood smear counts do, several groups have asked whether a PCR-derived measure of parasite load predicts the trajectory of an individual infection, not merely its presence. This review synthesizes the evidence on PCR-based detection and quantification across three connected questions: how PCR performs against microscopy and rapid diagnostic tests as a surveillance tool; whether parasite loads recovered by PCR carry prognostic information for disease severity; and what the accumulated submicroscopic-infection literature implies for using PCR as a routine surveillance instrument in both endemic and elimination settings.

2. Methods

We synthesized peer-reviewed studies addressing PCR-based detection and quantification of *Plasmodium* species, drawing on assay-development and validation reports, field comparisons of microscopy, rapid diagnostic tests and PCR, systematic reviews and meta-analyses of submicroscopic infection, and clinical studies relating parasite DNA load to disease severity. Sources were identified through the published malaria-diagnostics and surveillance literature spanning 1993 through 2018 and were selected for direct relevance to assay performance, prognostic value, or surveillance application; case reports and non-peer-reviewed material were excluded. Because the available literature is heterogeneous in population, transmission setting and assay chemistry, this review is a narrative synthesis rather than a pooled quantitative meta-analysis, and comparative figures are reported as given in the source study rather than re-derived.

3. Results

Diagnostic performance of PCR relative to microscopy and rapid diagnostic tests

Field comparisons consistently find that PCR recovers infections that both microscopy and antigen-based rapid tests miss. In a stratified survey conducted in Equatorial Guinea using semi-nested multiplex PCR as the reference

standard, 19.4% of PCR-positive samples were reported as negative by microscopy and 13.3% were missed by rapid diagnostic testing, figures that place both frontline tools within a broadly similar false-negative range even though they rely on entirely different detection principles [7] (Table 1). The same pattern holds for *Plasmodium vivax*, where a systematic review of forty studies found that the prevalence of infection detected by microscopy was, on average, 67% lower than the prevalence detected by PCR, meaning that population surveys relying on blood film alone substantially undercount vivax carriage [8] (Figure 1). Comparable findings emerged from a mixed-species endemic area of Thailand, where nested PCR identified a materially larger fraction of asymptomatic carriers than microscopy examined in parallel on the same samples [9], and from Papua New Guinea, where molecular methods recovered co-infections with more than one *Plasmodium* species that morphological examination of the same slides had scored as single-species [10].

Table 1: False-negative rates of microscopy and rapid diagnostic testing against a PCR reference standard, and the corresponding under-detection of *Plasmodium vivax* by microscopy relative to PCR in a pooled multi-study review [7,8].

Method	Reference standard	False-negative / under-detection	Study setting
Microscopy	Semi-nested multiplex PCR	19.4% false-negative	Equatorial Guinea [7]
Rapid diagnostic test	Semi-nested multiplex PCR	13.3% false-negative	Equatorial Guinea [7]
Microscopy (<i>P. vivax</i>)	PCR, pooled across studies	Prevalence ~67% lower than PCR	Systematic review, 40 studies [8]

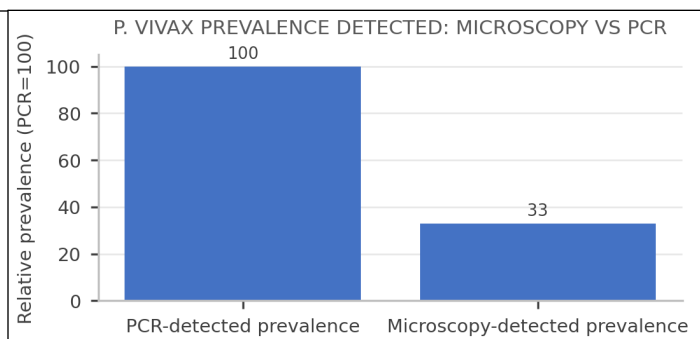


Figure 1: Relative prevalence of *Plasmodium vivax* infection detected by microscopy versus PCR, expressed with PCR-detected prevalence set to 100; a systematic review of forty studies found microscopy-detected prevalence averaged 67% lower than PCR-detected prevalence across surveyed populations [8].

The gap is not confined to low-density or asymptomatic carriage. Species misidentification by microscopy is itself a recurring problem: the zoonotic parasite *Plasmodium knowlesi* was repeatedly misread as *P. malariae* on blood film in Malaysian Borneo until PCR-based species typing resolved a large focus of naturally acquired human infection and, subsequently, confirmed knowlesi malaria as a cause of severe and occasionally fatal disease in adults previously assumed to have a benign quartan infection [11,12]. That episode is a useful illustration of the broader argument for PCR-based surveillance: it is not only more sensitive at the margin, it corrects errors of kind, not just errors of degree.

Parasite density, submicroscopic infection and prognostic significance

Because PCR returns a quantity rather than a threshold call, it opens the possibility of using parasite load itself as a prognostic variable, and the clearest evidence for this comes from severity studies that measured circulating parasite DNA directly. In a combined analysis of 435 patients drawn from African children and Asian adults with falciparum malaria, plasma concentrations of parasite DNA were approximately 40-fold higher in children with severe disease than in those with uncomplicated malaria, and approximately 10-fold higher in the corresponding comparison among adults, with diagnostic accuracy for severity comparable to plasma

histidine-rich protein 2 concentration and clearly superior to peripheral blood parasitemia alone [13] (Figure 2). This finding is consistent with the sequestration biology underlying severe falciparum malaria: mature parasitized erythrocytes adhere to microvascular endothelium and are depleted from the circulating pool that a blood film samples, so peripheral parasitemia systematically underestimates true parasite burden in exactly the patients for whom that burden matters most, whereas circulating parasite DNA, released continuously from the sequestered biomass, tracks total burden more faithfully [14].

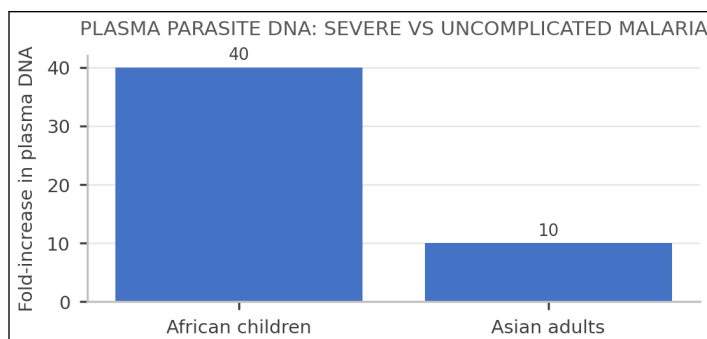


Figure 2: Fold-increase in plasma *Plasmodium falciparum* DNA concentration in severe versus uncomplicated malaria, in a combined analysis of 435 patients comprising African children and Asian adults, approximately 40-fold higher in children and approximately 10-fold higher in adults [13].

At the opposite end of the density spectrum, the same quantitative sensitivity that reveals a high-burden severe infection also reveals a very-low-burden submicroscopic one. A systematic review and meta-analysis across endemic-area surveys found that the proportion of PCR-detectable infections invisible to microscopy rises substantially as community-level transmission intensity falls, so that submicroscopic carriage becomes proportionally more important, not less, in the lower-transmission settings where elimination programs are concentrated [15]. A companion analysis modeling the transmission consequences of that finding estimated that submicroscopic carriers, despite lower individual gametocyte densities, can still account for a meaningful share of onward transmission in such settings because

they are numerically so much more common than microscopically patent infections [16]. Taken with the severity-end findings, this positions quantitative PCR as informative across the full clinical range, from carriers with too little parasite biomass for a blood film to register to patients whose parasite DNA load signals impending organ dysfunction.

Quantitative and real-time PCR platforms for surveillance

The translation of nested PCR into real-time and quantitative formats has been central to making molecular detection practical at surveillance scale. Real-time assays targeting the 18S ribosomal RNA genes of *P. falciparum*, *P. vivax* and *P. ovale* were validated for routine diagnostic use in the mid-2000s, reporting analytical sensitivity in the low single digits of parasites per microliter alongside same-day turnaround incompatible with the original gel-based nested protocol [17], and a parallel real-time platform validated against microscopy and nested PCR in a clinical laboratory setting confirmed comparable species-level discrimination with a substantially shorter processing time [18] (Table 2). High-throughput adaptations subsequently extended these real-time platforms to the sample volumes surveillance programs require, validating filter-paper and dried-blood-spot workflows capable of processing population-survey numbers of samples while still resolving very low parasite densities [19], and a further refinement targeting multi-copy subtelomeric sequence rather than single-copy 18S targets pushed analytical sensitivity roughly an order of magnitude beyond standard quantitative PCR, detecting infections at densities close to the theoretical limit imposed by blood sample volume itself [20]. Complementary molecular-marker work has continued to refine species-specific and genus-specific targets to improve throughput without sacrificing the specificity nested PCR established in the 1990s [21].

PCR-based surveillance in elimination programs

Table 2: Approximate analytical sensitivity by malaria detection method, from published assay-validation and diagnostic-comparison studies [1,6,17,18,20].

Method	Approximate detection limit	Turnaround	Source
Light microscopy (thick film)	~50-100 parasites/uL	20-60 min	[1]
Antigen-based RDT	~100-200 parasites/uL	15-20 min	[1]
Nested PCR (18S rRNA)	~1-5 parasites/uL	Hours (gel-based)	[6]
Real-time quantitative PCR	~1-2 parasites/uL	Same day	[17,18]
Ultrasensitive qPCR (multi-copy target)	Sub-parasite/uL, near sample-volume limit	Same day, batched	[20]

The practical case for PCR-based surveillance is strongest in the low-transmission settings where national programs are pursuing elimination, precisely because that is where the submicroscopic reservoir is largest relative to the patent, symptomatic caseload that routine facility-based reporting captures. Reviews of point-of-care diagnostics in elimination settings have concluded that rapid tests, however useful for individual case management, cannot reliably find the residual low-density infections that sustain transmission once clinical incidence has fallen, and that molecular surveillance is needed to characterize the reservoir those programs are trying to close [22]. Modeling work quantifying what a step-change in point-of-care sensitivity would achieve found that even a substantially more sensitive next-generation rapid test would still miss a meaningful share of the infectious reservoir in low-transmission settings, underscoring that PCR-level sensitivity, not merely an incremental antigen-test improvement, is what elimination surveillance actually requires [23]. Brief clinical reviews of malaria diagnosis have likewise noted that as elimination progresses, the operationally relevant question shifts from "is this patient infected" to "how much of the parasite reservoir remains undetected," a question only quantitative molecular tools are positioned to answer [24].

4. Discussion

The evidence reviewed here supports two related but distinct claims about PCR in malaria: it is a materially more sensitive diagnostic tool than microscopy or antigen-based rapid tests, and, separately, the parasite quantity it returns carries prognostic information that neither of those simpler tools can provide. The first claim is well established and largely uncontroversial; PCR's added sensitivity over microscopy and rapid diagnostic tests is documented across settings, species, and now several assay generations, from the original nested protocols of the early 1990s to var-gene-targeted ultrasensitive qPCR two decades later [6,20]. The second claim, that PCR-derived parasite load functions as a prognostic marker, is better supported for severe disease than for asymptomatic or submicroscopic infection, where the association is with transmission potential rather than individual outcome. The plasma-DNA severity data are among the more compelling findings in this literature precisely because they show a graded, biologically plausible relationship, tracking known sequestration pathophysiology, rather than a threshold effect [13,14].

Several limitations temper how far PCR-based surveillance can be pushed as a routine tool today. Turnaround time and infrastructure requirements remain real constraints outside reference and research laboratories; even the high-throughput adaptations validated for population surveys assume access to thermal cyclers, reliable reagent supply chains, and technical staff that many peripheral health facilities in endemic countries do not have [19]. Cost per sample, while falling, still exceeds that of a rapid diagnostic test by a wide margin, which matters when a surveillance program must screen thousands of asymptomatic community members to find a comparatively small submicroscopic reservoir. There is also a conceptual limitation worth stating plainly: detecting circulating parasite DNA is not the same as detecting viable, transmissible parasites, and at the very low densities where PCR's advantage over microscopy is

largest, the biological significance of a positive result for either individual prognosis or onward transmission is least well characterized. The knowlesi episode is a useful corrective here too, since it shows PCR's diagnostic value is not limited to sensitivity at low density; correcting species misclassification at ordinary, microscopically patent densities changed clinical management for a syndrome that had been mistaken for something far more benign [12].

Taken together, the literature supports a tiered role for PCR rather than a wholesale replacement of microscopy or rapid diagnostic testing. For individual clinical management in resource-limited settings, microscopy and antigen tests remain appropriate first-line tools, with PCR reserved for cases where species identification is clinically consequential, such as suspected knowlesi infection or discordant results in a returning traveler, and, increasingly, as a severity-relevant quantitative measure in research and reference-hospital settings. For population-level surveillance, particularly in the low-transmission settings that elimination programs prioritize, the case for molecular methods is considerably stronger, since the reservoir those programs must characterize is disproportionately submicroscopic and therefore effectively invisible to any tool with a higher detection floor.

5. Conclusion

PCR-based methods have matured from a species-confirmation tool into a quantitative surveillance and, in the setting of severe disease, prognostic instrument, and the accumulated evidence supports both roles rather than a single narrow use case. The sensitivity advantage over microscopy and rapid diagnostic testing is large and consistent, the parasite-load information it returns tracks disease severity in a biologically coherent way, and the submicroscopic reservoir it alone can reliably characterize is exactly the target elimination programs need to see. Realizing that potential at scale still

depends on solving the infrastructure and cost barriers that keep routine PCR out of most peripheral health facilities, but the diagnostic and prognostic case for continued investment in molecular surveillance is, on the present evidence, a strong one.

6. Acknowledgements

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8. Conflict of Interest

The authors declare no conflict of interest relevant to the content of this review.

9. Author Contributions

RC conceived the review and drafted the manuscript. BC performed the literature synthesis on diagnostic performance and surveillance applications. RG contributed the analysis of severity and prognostic data and critically revised the manuscript. All authors read and approved the final version.

10. Ethics Statement

This article is a narrative literature review of previously published studies and did not involve new data collection from human or animal subjects; institutional ethics approval was therefore not required.

11. Data Availability

No new data were generated for this review. All findings discussed are drawn from the cited, publicly available published literature.

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