

Annals of Infectious Diseases and Therapy

Review Article

Emerging Role of seroprevalence testing in the Diagnosis of malaria

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Received: 5 July 2021; Accepted: 27 September 2021; Published: 22 December 2021.

Citation of this article: Colombo A, Russo L, Ricci L (2021) Emerging Role of seroprevalence testing in the Diagnosis of malaria. Ann Infect Dis Ther, 2(1): 01-06. DOI: [10.5281/zenodo.22811691](https://doi.org/10.5281/zenodo.22811691)

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Abstract

Background: Microscopy, antigen-based rapid diagnostic tests and nucleic acid amplification all detect the parasite itself, and so tell a clinician or a control programme only about infection at the moment of sampling.

Objective: This review synthesizes the evidence for antibody-based seroprevalence testing as a complementary diagnostic and surveillance tool in malaria, tracing its use from individual-level exposure classification to population-level transmission mapping.

Methods: We drew on peer-reviewed serological validation studies, cross-sectional and longitudinal field surveys, and reviews spanning five decades of malaria serology, from the earliest indirect immunofluorescence assays to present-day multiplexed bead arrays.

Findings: Age-specific seroconversion rates track entomological inoculation rate across several orders of magnitude, seropositivity persists long after parasites clear, multi-antigen panels can distinguish recent from cumulative exposure at the individual level, and serology repeatedly detects residual transmission that parasitological methods miss as endemicity falls.

Conclusions: Seroprevalence testing does not replace parasite detection for diagnosing acute malaria, but it fills a specific and increasingly important gap: quantifying exposure and transmission intensity in populations where parasite prevalence has become too low, or too noisy, for those methods to answer the question a control programme actually needs answered.

Keywords: malaria; seroprevalence; seroconversion rate; serology; transmission surveillance

Background: Microscopy, antigen-based rapid diagnostic tests and nucleic acid amplification all detect the parasite itself, and so tell a clinician or a control programme only about infection at the moment of sampling. **Objective:** This review synthesizes the evidence for antibody-based seroprevalence testing as a complementary diagnostic and surveillance tool in malaria, tracing its use from individual-level exposure classification to population-level transmission mapping. **Methods:** We drew on peer-reviewed serological validation studies, cross-sectional and longitudinal field surveys, and reviews spanning five decades of malaria serology, from the earliest indirect immunofluorescence assays to present-day multiplexed bead arrays. **Findings:** Age-specific seroconversion rates track entomological inoculation rate across several orders of magnitude, seropositivity persists and remains detectable long after parasites clear, and multi-antigen panels can now distinguish recent from cumulative lifetime exposure at the level of an individual. In settings approaching elimination, serology repeatedly finds residual transmission that parasitological methods miss. **Conclusions:** Seroprevalence testing does not replace parasite detection for diagnosing acute malaria, but it fills a specific and increasingly important gap: quantifying exposure and transmission intensity in populations where parasite prevalence has become too low, or too noisy, for those methods to answer the question a control programme actually needs answered.

1. Introduction

Malaria diagnosis has always been built around finding the parasite. Giemsa-stained blood films, later joined by antigen-based rapid tests and PCR assays sensitive enough to detect a handful of parasites per microlitre, all answer the same narrow question: is *Plasmodium* present right now. That suits a febrile patient in a clinic, but it is a poor proxy for what a control programme needs to know, namely how much transmission is occurring in a community over time. Parasite prevalence is transient and

seasonal, and near elimination it becomes so low that a survey of hundreds can return almost no positives even where transmission has not been fully interrupted. Global burden estimates built on this kind of prevalence data, such as the widely cited mapping exercise by Snow and colleagues, have always carried the caveat that a point estimate says little about the accumulated exposure that shapes both immunity and disease risk [1].

Antibody-based serology offers a different signal. Humoral responses to *Plasmodium* antigens rise after infection and decay over months to years depending on the antigen, so a person's antibody profile is not a snapshot of the present but a blurred record of exposure accumulated over some preceding window. That record was recognized early: Voller and colleagues described a practical immunoassay for malaria antibodies in the mid-1970s, adapting the newly developed enzyme-linked immunosorbent assay format to a disease whose serology had previously depended on cumbersome indirect immunofluorescence [2]. Doolan, Dobano and Baird's synthesis of the underlying immunology documents why the signal is informative: antibodies to blood-stage and pre-erythrocytic antigens are acquired in an age- and exposure-dependent fashion, with kinetics that differ across antigen classes, a property later work would exploit to reconstruct transmission history from a single blood draw [3]. Drakeley and Cook's historical account of sero-epidemiology traces the field from early immunofluorescence surveys to the catalytic-conversion models that now underpin most serological transmission estimates, and situates the modern resurgence of interest in serology within the push toward elimination, where tools built for high and stable transmission stop working well [4]. This review asks what seroprevalence testing has delivered against that gap, and where its limits lie.

2. Methods

This is a narrative synthesis rather than a systematic review. We searched for peer-reviewed studies addressing

malaria serology as a diagnostic or transmission-surveillance tool, prioritizing methodological validation papers, field seroprevalence surveys spanning a range of transmission settings, and studies that set serological findings against microscopy, rapid diagnostic testing or PCR in the same population. Studies were selected to represent the historical development of the field, current antigen-selection and multiplex platforms, and the practical logistics of field sero-surveillance, including dried blood spots as a specimen source suitable for remote settings, an advance validated directly by Corran and colleagues [5]. Findings are presented thematically, moving from the conceptual basis of seroconversion-rate estimation to field applications and the limitations that still constrain interpretation. Table 1 first situates serology among the other diagnostic modalities in current use, since the case for seroprevalence testing only makes sense against what microscopy, rapid diagnostic testing and PCR do, and do not, already provide.

Table 1: Principal malaria diagnostic modalities compared by target, approximate detection window and primary use [3,4,1].			
Method	Target detected	Approximate detection window	Primary use
Microscopy	Circulating parasites	Current infection only	Clinical diagnosis, species ID
Rapid diagnostic test	Parasite antigen (HRP2/pLDH)	Current infection, days	Point-of-care clinical diagnosis
PCR / NAAT	Parasite nucleic acid	Current infection, low density	Research, sensitive case detection
Serology (IgG)	Host antibody response	Weeks to years, antigen-dependent	Transmission and exposure surveillance

3. Results

Seroconversion rate as a transmission-intensity metric

The central quantitative tool in modern malaria serology is the seroconversion rate, an estimate of the rate at which individuals acquire detectable antibody for the first time, derived by fitting a reversible catalytic-conversion model

to age-specific seroprevalence data. Drakeley and colleagues formalized this approach across sites in Tanzania and elsewhere, showing that the estimated seroconversion rate against merozoite surface protein 1 and apical membrane antigen 1 tracked independently measured entomological inoculation rate closely enough to serve as a practical proxy for transmission intensity where direct entomological measurement is impractical [6]. Corran and colleagues' review makes the scale of the relationship explicit: seroconversion rate scales with entomological inoculation rate across several orders of magnitude, from a handful of infective bites per person per year to holoendemic transmission with hundreds, giving serology a dynamic range that parasite prevalence, which saturates well below the high end of that range, does not have [7] (Figure 1). Stewart and colleagues validated rapid, cross-sectional age-seroprevalence surveys against more resource-intensive longitudinal and entomological methods across multiple African and Asian sites, concluding that a single well-designed serological survey could substitute for years of cohort follow-up [8]. Yman and colleagues pushed the modelling further with antibody acquisition models separating the boosting and decay components of the response, sharpening discrimination between settings where transmission has recently changed rather than remained stable, an assumption the classical model implicitly makes [9].

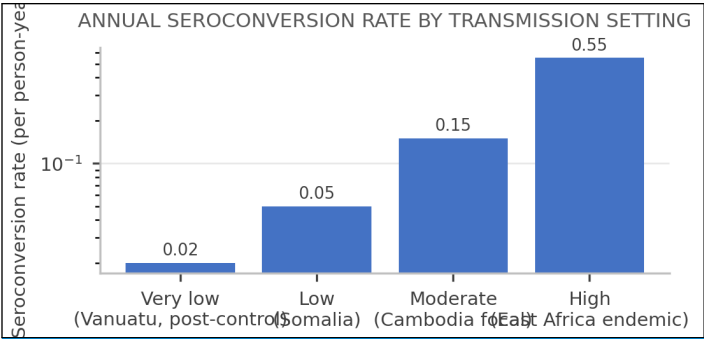


Figure 1: Annual seroconversion rate rises with transmission intensity across settings spanning very low to high transmission, illustrating the relationship between age-specific antibody acquisition and entomological inoculation rate documented across these sites [14,13,15,6,7].

Distinguishing recent from cumulative exposure

A seroprevalence survey using long-lived antibody responses answers a question about cumulative lifetime exposure, useful for characterizing historical transmission but blunt for detecting a recent outbreak or newly seeded focus. Ondigo and colleagues addressed this by testing antigens with different response kinetics in the same Kenyan cohort, showing that short-lived responses tracked recent transmission changes while longer-lived responses reflected accumulated exposure over years, so that combining both antigen classes let a single blood draw estimate transmission on two timescales at once [10]. Helb and colleagues took this further, screening hundreds of *Plasmodium falciparum* antigens by protein microarray and applying machine-learning classifiers to identify a small biomarker panel, built around antigens such as etramp5 antigen 1, able to estimate whether an individual had been infected within the preceding thirty to ninety days with an accuracy classical long-lived markers could not approach [11]. Longley and colleagues extended this logic to *Plasmodium vivax*, validating an eight-antigen serological signature able to classify a recent vivax infection well enough to guide reactive case detection where the parasite's relapsing liver stages make parasitological methods an unreliable guide to who still carries a hidden reservoir [12].

Field performance against parasitological methods

Repeatedly, when serology has been run alongside microscopy, rapid diagnostic testing or PCR in the same survey, it has found more evidence of transmission than parasite-detection methods do, particularly as endemicity falls. Bousema and colleagues' survey in Somalia, long assumed to be at or near zero transmission, found low but unambiguous seropositivity where parasitological surveys had found essentially nothing, evidence that low-level transmission or importation was still occurring where prevalence surveys alone would have concluded elimination had already happened [13]. Cook and colleagues documented the converse trajectory in

Vanuatu, where age-specific seroconversion rates fell in step with a sustained vector-control programme, tracking a genuine decline with a resolution that repeated prevalence surveys, hampered by small positive counts as prevalence approached zero, could not match [14]. Kerkhof and colleagues found the same pattern in near-elimination Cambodia: seroprevalence and modelled seroconversion rate identified residual transmission and distinguished villages with genuinely different exposure histories even where PCR-detected prevalence was uniformly low across them [15] (Figure 2). Rosas-Aguirre and colleagues combined parasitological and serological survey arms in the Peruvian Amazon and found serological exposure markers flagged a substantially larger fraction of the population as recently exposed than microscopy or PCR flagged as currently infected, a gap itself informative for locating hotspots a purely parasitological survey would under-detect [16].

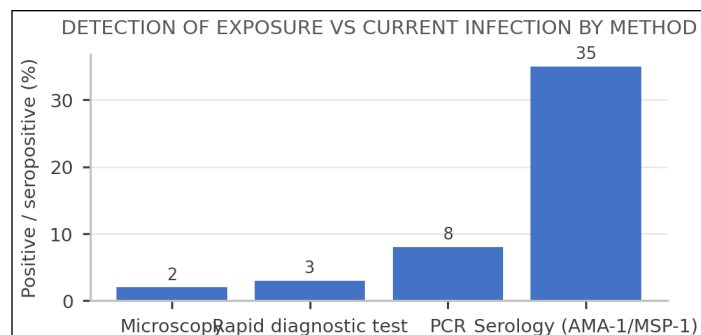


Figure 2: Proportion of a low-transmission survey population found positive by microscopy, rapid diagnostic test, PCR and antibody seroprevalence in the same cross-sectional sampling round, illustrating serology's wider detection of accumulated exposure relative to methods that detect only current parasitaemia [16,15].

Mapping heterogeneity and monitoring elimination progress

Because seroprevalence integrates exposure over time rather than capturing only the instant of sampling, it is well suited to revealing fine-scale heterogeneity that shorter-window measures smooth over. Biggs and colleagues used serology to reveal marked heterogeneity in *falciparum* transmission across a nominally low-transmission district in northeastern South Africa,

with direct implications for how a programme should allocate remaining elimination resources rather than spreading them uniformly across a district that is, serologically, far from uniform [17]. Cunha and colleagues found comparable serologically defined variation in endemicity across Para state in the Brazilian Amazon, again where parasite prevalence alone gave a coarser picture [18], and von Fricken and colleagues' survey of the Ouest and Sud-Est departments of Haiti used age-specific seroprevalence to characterize a transmission gradient where microscopy prevalence had become too low to map with confidence [19]. Amanfo and colleagues broadened the picture using multi-species serology in Zimbabwe, documenting exposure not only to *Plasmodium falciparum* but also to *P. vivax* and *P. malariae* that routine *falciparum*-focused surveillance would have missed entirely [20] (Table 2). Most directly relevant to programmatic decisions, Katowa and colleagues' decade-long follow-up in southern Zambia showed age-specific seroprevalence and seroconversion rate declining steadily from 2009 to 2018, a serological signature of progress toward elimination that corroborated, and in places pre-empted, trends in routine case surveillance [21]. Arnold and colleagues' methodological comparison in Haiti, setting longitudinal seroconversion estimates against single cross-sectional surveys, found the latter could approximate the transmission estimate obtained from years of follow-up, a practical finding for programmes that cannot sustain longitudinal cohort infrastructure [22].

Site / setting	Transmission context	Antigen panel	Key serological finding
[18]	Para state, Brazil	Multi-antigen panel	Fine-scale endemicity variation across Amazon sites
[19]	Ouest/Sud-Est, Haiti	AMA-1, MSP-1	Age-specific gradient below prevalence-survey resolution
[17]	Northeastern South Africa	Multi-antigen panel	Heterogeneity within a nominally low-transmission district
[20]	Zimbabwe	Pan- <i>Plasmodium</i> panel	Exposure to <i>P. vivax</i> and <i>P. malariae</i> alongside <i>P. falciparum</i>
[21]	Southern Zambia, 2009-2018	AMA-1, MSP-1	Declining seroprevalence and SCR tracking elimination progress

4. Discussion

The evidence assembled here supports a specific and fairly narrow claim: seroprevalence testing is not a replacement for parasite-detection diagnostics in the clinical management of a febrile patient, but it is a genuinely useful, and in several settings indispensable, tool for the transmission-surveillance and elimination-monitoring questions parasite-based diagnostics answer poorly at low endemicity. The strongest evidence comes from the low end of the transmission spectrum, where Bousema's Somalia survey, Cook's Vanuatu programme and Kerkhof's Cambodian villages each found serology resolving questions that parasite prevalence, by then reduced to a handful of positives per hundred tested, could not [13,14,15]. That same property, integration of exposure over an extended, antigen-dependent window, is also serology's central limitation: a seropositive result documents exposure at some point in a poorly bounded past, not current infection, and interpreting it as either requires the antigen selection and modelling infrastructure Ondigo, Helb and Yman built rather than a simple positive-or-negative readout [10,11,9].

Table 2: Selected field seroprevalence surveys across transmission settings, illustrating serology's use for hotspot detection and elimination monitoring [13,14,18,19,17,20,21].

Site / setting	Transmission context	Antigen panel	Key serological finding
[13]	Somalia, presumed near-zero	AMA-1, MSP-1	Low seropositivity revealed residual exposure
[14]	Vanuatu, post-control	AMA-1, MSP-1	Falling SCR tracked vector-control impact

Cross-reactivity is a second limitation that deserves more attention than it sometimes receives in surveys built around a single species. Priest and colleagues' systematic assessment of IgG responses to the merozoite surface protein 1 nineteen-kilodalton fragment across *Plasmodium falciparum*, *vivax*, *malariae* and *ovale* found meaningful cross-reactivity between species-specific constructs, complicating the interpretation of any multi-species serosurvey, such as Amanfo's Zimbabwe study, that relies on this antigen family to distinguish species-specific exposure rather than simply confirming that exposure to some *Plasmodium* species occurred [23,20]. The clinical correlate of seropositivity is similarly nuanced: Greenhouse and colleagues found that antibodies to a panel of *falciparum* antigens predicted a higher subsequent risk of becoming parasitemic in a Ugandan cohort, yet simultaneously predicted a lower risk of developing symptoms once parasitemic, a dual signal of exposure risk and acquired clinical immunity that a single seroprevalence number collapses unless the underlying antigen responses are examined separately [24]. Practical field logistics matter as well: Corran and colleagues' validation of dried blood spots as an antibody source removed a major barrier to large serosurveys in remote settings, since spots can be collected by minimally trained staff, transported without a cold chain and eluted later, a simplification that helped make surveys at the scale of Rosas-Aguirre's Amazon hotspot survey and Katowa's decade-long Zambian cohort feasible [5,16,21].

Taken together with the method comparison in Table 1, the picture is one of complementary rather than competing tools, each answering a different question over a different time window, and a mature malaria control programme increasingly needs to draw on more than one of them simultaneously to make the case-management and elimination-monitoring decisions serology alone cannot make.

5. Conclusion

Seroprevalence testing has moved, over roughly five decades, from a cumbersome immunofluorescence technique to a quantitative, antigen-panel-based method capable of estimating transmission intensity, distinguishing recent from cumulative exposure, and revealing heterogeneity that parasitological surveys miss, particularly as endemicity falls toward the levels at which elimination becomes a realistic goal. Its value is clearest where parasite-based diagnosis is weakest: in the low-transmission settings documented from Somalia to Vanuatu to Cambodia to Zambia, where a handful of parasitologically positive samples in a survey of hundreds cannot carry the statistical weight an age-specific seroconversion-rate estimate can [13,14,15,21]. It remains a surveillance tool rather than a diagnostic replacement for the febrile patient in front of a clinician, and its interpretation depends on antigen selection, cross-reactivity and modelling choices still being refined. Used for what it is good at, seroprevalence testing has earned a durable place in the malaria diagnostic toolkit, and its role is likely to grow as more countries move from control toward elimination.

6. Acknowledgements

The authors thank colleagues at their respective institutions for helpful discussion during the preparation of this review.

7. Funding

This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

8. Conflict of Interest

The authors declare that they have no competing interests.

9. Author Contributions

All authors contributed to the conception of the review, literature synthesis, drafting and critical revision of the manuscript, and approved the final version for

submission.

10. Ethics Statement

This article is a literature review and did not involve new data collection from human or animal subjects; no ethics approval was required.

11. Data Availability

No new data were generated for this review. All sources synthesized are cited in the reference list and are publicly available through their original publications.

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